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COMPOSITION OF RHUBARB AT DIFFERENT STAGES OF MATURITY IN RELATION TO ITS USE IN COOKING AND CANNING[1]

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REVIEW OF LITERATURE

There is considerable literature dealing with the composition of different portions of the rhubarb plant. Much of this concerns the chemistry of the root and the isolation and identification of the constituents responsible for its therapeutic effects. In the present investigation the composition of the root is of interest because limited quantities of the therapeutically active substances may be present in the petioles and practically all the food constituents of the petiole occur to some extent in the root. The literature on the root is summarized in the standard text books of pharmacology, but particular mention may be made of the work of Tutin and Clewer (*32*),[3] who identified many of the constituents of the root and cleared up much of the confusion in the terminology of this subject. Of special importance also is the work of Müller (*22*), who has isolated certain substances occurring in the root.

Because of the possibility that oxalic acid may occur in quantities large enough to be toxic, considerable work has been done on the nature and amounts of the organic acids of the rhubarb plant and on the toxicity of the oxalic acid as well as that of different parts of the plant itself. The results of these investigations (*1, 2, 3, 6, 10, 16, 19, 21, 23, 24, 27, 28, 31*) show that malic acid predominates in the petiole and that citric acid and oxalic acid occur in much smaller quantities. The total acidity appears to range from 1 to 2 per cent. It is also shown that generally there is not sufficient oxalic acid in the petioles, or leafstalks, to cause harmful effects if they alone are eaten, but most investigators consider it unsafe to eat the leaves when cooked in the same way as spinach or other greens.

From the reports of several investigators (*5, 7, 30*) on the food constituents of rhubarb, it is evident that the food value is rather low and that the constituents vary considerably.

Several investigators have given attention to the ash constituents of rhubarb. From the work of Sherman (*30*) it appears that with the exception of potassium the mineral constituents are low.

The vitamin content of rhubarb has been studied by Pierson and Dutcher (*26*) and by Hessler and Williams (*15*). Their papers are of interest in the present investigation because they deal to some extent with the methods of preparation and preservation.

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[2] The material used in this investigation in 1928 was kindly furnished by D. N. Shoemaker, of the Division of Horticultural Crops and Diseases.

[3] Reference is made by number (italic) to Literature Cited, p. 400.

Cruess (*9*) states that rhubarb when canned has an intensely corrosive action upon the tin container and causes swelling and sometimes even perforations. The reports of other investigators (*8*, *17*, *18*, *29*) show a lack of agreement, which may be partly due to differences in the material used.

OBJECT OF THE INVESTIGATION

Investigators have found considerable variation in the composition of rhubarb, in its properties when cooked, and in its behavior when canned. The main object of the present investigation was to determine the effect of age on the composition of rhubarb and the relation of its composition to its suitability for cooking and canning. It was thought advisable to determine also whether seasonal conditions affect its composition. The practical canning and cooking tests were accompanied by a study of the biochemistry of the development of the rhubarb petiole and leaf blade and its relation to seasonal conditions. The results of this phase of the investigation are reported in detail elsewhere (*10*).

SOURCE OF MATERIAL

The material used in the canning and cooking tests in 1926 and 1927 was purchased in the market at Washington, D. C. The appearance of the material indicated that it was all of the same variety, either Victoria or a strain closely resembling it. The material was fresh and at a stage of maturity typical of that generally offered for sale in the markets. The leafstalks were 15 to 18 inches long and weighed 60 to 80 g each.

The material used in 1928 was grown in the test plots at the Arlington Experiment Farm, Rosslyn, Va. The variety was Ruby, a seedling of Victoria (*Rheum hybridum* Murr.), introduced by the Central Experimental Farm, Ottawa, Canada, in 1923 (*20*). This variety was recommended because of the freedom of its leafstalks from stringiness and extreme acidity. The leafstalks are not particularly large but are very numerous and generally contain more than the usual quantity of the red anthocyan pigment in the epidermis. The divisions were obtained from 2-year-old crowns grown in the immediate vicinity of the present planting, which was made in the spring of 1927, the method used being that ordinarily employed in propagating rhubarb. All the chemical analyses reported in this paper were made upon the material grown on the Arlington Experiment Farm in 1928.

SOIL AND CULTURE

The soil on which the rhubarb was grown was a moderately fertile loam to which had been added generous quantities of well-rotted manure. In the fall of 1927 the plants were mulched with barnyard manure, but in 1928 no fertilizer or manure was applied until after the last samples were taken for analysis. The plants were given frequent hoeing throughout the year to remove weeds.

METHODS OF SAMPLING AND ANALYSIS

Four series of samples of known age were secured in the season of 1928. The leaves were just beginning to make their appearance

aboveground on April 10. On April 21 the petioles were 2 to 4 inches long and the leaves in many cases had not completely unfolded. On this date a large number of petioles were marked by means of tags, so that leaves of known age could be collected at any time desired. Six collections made at intervals of 7 to 12 days and extending up to June 15 constitute the first series. While this series was being taken the plot was gone over at intervals of 3 to 6 days and the new leaves were tagged as the petioles became 2 to 4 inches long; this treatment was continued to October 9. The leaves tagged in April were considered to be about 10 days old; those tagged in June and July about 5 days old. The higher temperatures of June and July caused more rapid development, so that samples of a given age taken in June or July were at a somewhat later stage of growth than those of like age taken in April or May. From the various lots of tagged leaves, three later series, each consisting of petioles 5 to 60 days old, were collected on June 5, July 6, and October 17, respectively. Broken or injured leaves were always discarded. The leaves were picked by pulling them from the crown, as is customary in harvesting rhubarb. The material for analysis was collected about 10 a. m., and 15 to 25 leaves were used to obtain each chemical sample. Thin cross sections were cut from near the middle of the petioles, and from these petioles 100-g samples were weighed out; enough 95 per cent alcohol was added to bring the concentration up to 75 to 80 per cent. The material was brought to boiling and sealed. For total nitrogen determinations similar samples were dried in a vacuum oven at 70° to 75° C.

The preserved samples were extracted with alcohol and made up to volume. Aliquot portions of these were used for the various determinations. The acid was titrated with N/10 NaOH and calculated as malic acid. The sugars were determined by the volumetric permanganate method recommended by the Association of Official Agricultural Chemists (*4*). The acid-hydrolyzable polysaccharides were determined upon the residue after extraction with alcohol. Total nitrogen was determined by the Kjeldahl method as modified to include nitrate nitrogen. Nitrate nitrogen was determined by the ferrous chloride method as recommended in the Methods of the Association of Official Agricultural Chemists (*4*) for nitrates in meats. The total astringency was determined by the Proctor-Loewenthal method (*25*).

ANALYTICAL RESULTS

The first series differed from the others in that the successive samples making it up were taken at 10-day to 12-day intervals over a period of 55 days, hence under environmental conditions that differed from sample to sample. Later, all the samples constituting a series were taken at one time, and hence had been subjected to identical conditions. Allowing for these differences, the general course of chemical change during development was, broadly speaking, the same in the four series, as is indicated by the similarity of the curves shown in Figures 1 to 6 and by the data given in Table 1.

TABLE 1.—*The composition of rhubarb petioles at different stages of development expressed in percentage of fresh green weight*

SERIES A

Date of sampling	Age	Length of petiole	Total solids	Solids soluble in alcohol	Solids insoluble in alcohol	Total sugars	Acid-hydrolyzable polysaccharides	Acids	Tannins	Nitrate nitrogen	Amino nitrogen	Total nitrogen
	Days	*Inches*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
April 21	10	2- 4	5.21	2.96	2.25	0.65	0.55	0.999	0.096	0.014	0.045	0.211
May 5	24	10-12	4.80	2.44	2.36	.30	.53	.948	.058	.030	.029	.203
May 19	38	12-14	5.71	2.80	2.91	.42	.67	.884	.086	.067	.027	.178
May 26	45	14-18	5.87	2.90	2.97	.60	.68	1.000	.109	.065	.036	.155
June 5	55	14-18	5.81	2.96	2.85	.67	.58	.818	.073	.077	.024	.127
June 15	65	14-18	6.52	2.96	3.56	.66	.70	.749	.110	.100	.020	.130

SERIES B

June 5	5	3- 4	5.33	2.84	2.49	0.40	0.54	1.080	0.122	0.024	0.043	0.198
	8	6-10	4.83	2.60	2.23	.39	.48	1.110	.109	.029	.045	.160
	11	10-12	4.60	2.48	2.12	.33	.46	1.080	.127	.030	.055	.136
	15	12-14	4.84	2.72	2.12	.39	.47	1.110	.096	.032	.040	.142
	19	14-16	4.56	2.56	2.00	.29	.42	1.070	.088	.035	.039	.149
	25	16-18	5.25	2.96	2.28	.52	.45	1.085	.091	.038	.030	.127
	32	18-21	5.81	3.16	2.65	.60	.58	1.140	.087	.044	.030	.134
	40	18-21	6.17	3.37	2.80	.90	.72	1.160	.079	.056	.026	.130
	55	14-18	5.81	2.96	2.85	.67	.58	.818	.073	.077	.024	.127

SERIES C

July 6	5	4-16	6.57	3.44	3.13	0.40	0.76	1.520	0.168	0.017	0.071	0.267
	9	10-12	6.45	3.44	3.01	.50	.79	1.580	.153	.022	.068	.228
	13	12-16	6.15	3.20	2.95	.60	.77	1.380	.123	.026	.057	.197
	28	18-24	7.19	3.60	3.59	1.05	.84	1.320	.201	.027	.043	.149
	42	20-28	7.43	3.48	3.95	.91	.78	1.150	.162	.053	.038	.148
	60	20-28	6.82	3.96	3.86	.56	.75	.781	.265	.073	.010	.121

SERIES D

October 17	8	9-14	6.29	3.56	2.73	1.04	0.63	1.560	0.111	0.023	0.070	0.201
	12	14-18	6.35	3.44	2.91	1.04	.64	1.430	.114	.026	.070	.186
	24	16-20	7.22	3.76	3.46	1.14	.74	1.300	.110	.026	.050	.180
	40	16-20	7.23	3.84	3.39	1.05	.72	1.280	.115	.030	.041	.159
	60	16-20	5.64	2.52	3.12	.58	.67	.934	.100	.045	.039	.132

SOLIDS

Table 1 shows the results of analyses expressed in percentages of the fresh green weight. A high moisture content for all the samples is shown by the low percentages of total solids, although these varied somewhat with the season in which the samples were taken. Early in the season the solids were below 5 per cent in some samples, whereas later in the season they were above 7 per cent in some samples. The curves for the total dry matter in the four series of samples (fig. 1) show that the total solids were generally lower in the series taken in April and June than in the series taken in July and October. The weather records show that the samples taken in April and May grew under conditions of shorter days, and hence with less sunshine and with a lower temperature. The relative intensity of the photosynthetic activity may account for some of the differences in the four series. Since the samples were taken in July and October after several

days in which no rain fell it might be assumed that the soil moisture had a considerable effect. These data indicate that a high percentage of solids may be expected in plants grown under conditions of low or medium soil moisture, high temperature, and long hours of sunshine.

The data in Table 1 show also that the percentage of solids varied somewhat with age. Very young petioles, 3 to 4 inches long, had a medium percentage of solids; this decreased during the next few days, then increased, and finally, with one exception, decreased in the very old samples. The petioles seem to have been at about their lowest in solids at the time they were picked for market, or a little earlier.

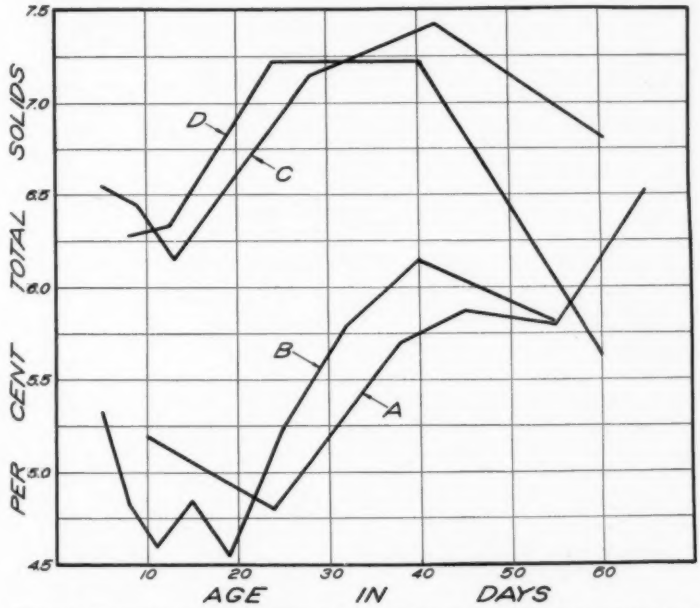

FIGURE 1.—Changes in total solids of rhubarb petioles with increase in age: A, Series collected April 21 to June 15; B, series collected June 5; C, series collected July 6; D, series collected October 17

About half the total solids were soluble and half insoluble in alcohol. Both soluble and insoluble solids were higher in the series taken in April and May. Insoluble solids varied with age, being lowest in the young samples and highest in the old ones. The soluble solids increased with age or remained nearly constant.

SUGARS

The total sugars were very low and showed no very significant change with age. However, the series taken on July 6 and on October 17 were slightly higher in sugars than those taken earlier. Figure 2 shows the curves for total sugars.

ACID-HYDROLYZABLE POLYSACCHARIDES

The acid-hydrolyzable polysaccharides were also very low. The material in these samples gave a negative iodine test for starch. Differences due to age are not very significant.

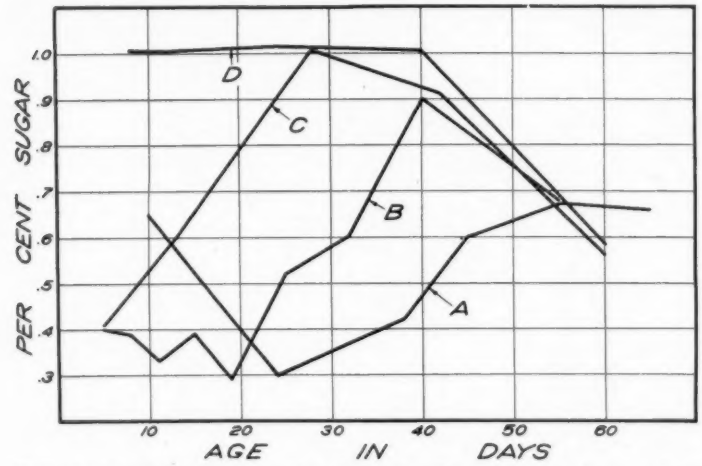

FIGURE 2.—Changes in total sugar content of rhubarb petioles with increase in age: A, Series collected April 21 to June 15; B, series collected June 5; C, series collected July 6; D, series collected October 17

ACIDS

One of the chief characteristics of rhubarb as a food material is its high acidity. In the variety studied, which is considered comparatively low or medium in acidity, the acid content calculated as malic acid on the fresh green-weight basis may be as high as 1.5 per cent. The high acid content, together with the very low sugar content, makes this variety extremely tart. Except in very old samples, the acidity is considerably higher in the series taken in July and October than in the series taken in April and May. The climatic conditions favorable to high acidity seem to be long hours of intense sunshine, medium or low rainfall, and high temperature. Rhubarb is often said to vary greatly in acidity. In many cases this may be due to varietal differences, but the present study shows that it may also be due to differences in stage of maturity or climatic conditions. The tendency already noted of the titratable acidity to decrease with age is not very noticeable until after the rhubarb has passed the stage in which it is in prime condition for table use. In very old samples the decrease is very marked. It is apparent from Figure 3 that the acidity may remain nearly constant during most of the period in which the material is in prime condition for table use.

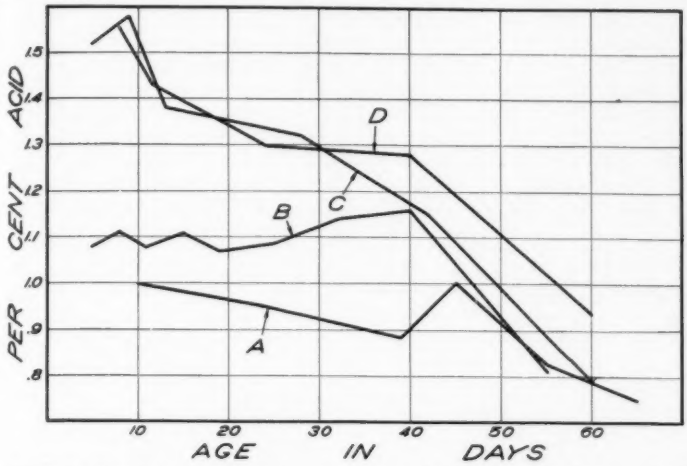

FIGURE 3.—Changes in titratable acidity of rhubarb petioles with increase in age: A, Series collected April 21 to June 15; B, series collected June 5; C, series collected July 6; D, series collected October 17

TOTAL NITROGEN

The total nitrogen in rhubarb is decidedly low, as it is in many fruits. The nitrogen content of the samples analyzed varied from 0.267 to 0.121 per cent. (Table 1.) If these percentages are multiplied by 6.25, the factor used for computing the percentage of protein,

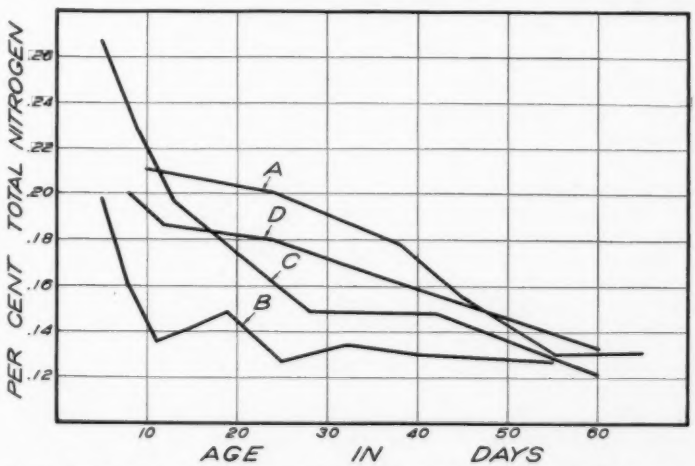

FIGURE 4.—Changes in total nitrogen content of rhubarb petioles with increase in age: A, Series collected April 21 to June 15; B, series collected June 5; C, series collected July 6; D, series collected October 17

it will be apparent that the protein content of rhubarb is often less than 1 per cent. The total nitrogen, highest in the young samples, decreases rapidly as the petiole develops and more slowly in the older stages. (Fig. 4.)

NITRATE NITROGEN

Changes in the content of nitrate nitrogen are of particular interest. Table 1 shows that in certain cases a high percentage of the total nitrogen is in the form of nitrates. The nitrate nitrogen was lowest in the young samples and increased steadily throughout the life of the leaf. (Fig. 5.) In one sample 77 per cent of the total nitrogen of the old petioles was in the form of nitrates. It may be inferred that the presence of such a high percentage of nitrate is significant as a factor in the corrosion of the metal of the tin container.

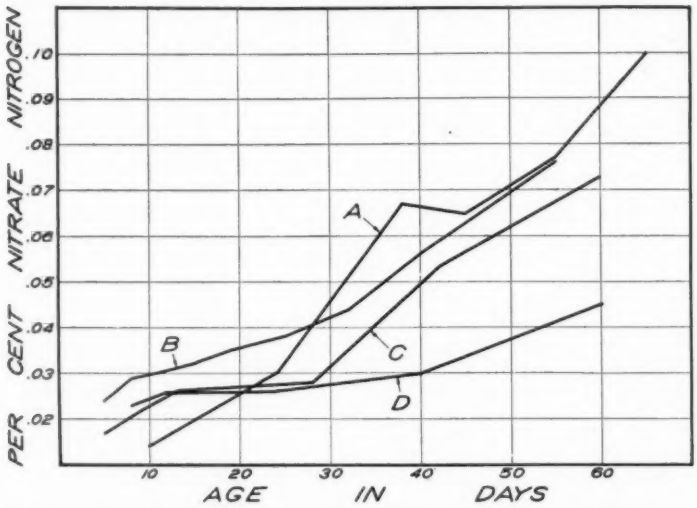

FIGURE 5.—Changes in nitrate nitrogen content of rhubarb petioles with increase in age: A, Series collected April 21 to June 15; B, series collected June 5; C, series collected July 6; D, series collected October 17

The series of samples taken in October was lower in nitrate nitrogen than those taken earlier. It seems probable that this was due to the reduction of available nitrates in the soil as the season advanced and that the variations in the different series were directly related to variations in the available nitrates in the soil. It would therefore be expected that soil conditions and fertilizer treatment would very materially affect the amount of nitrate nitrogen.

AMINO NITROGEN

In many cases a considerable portion of the total nitrogen is amino nitrogen. It was highest in the young material and decreased as the petiole grew older. Figure 6 shows the results for the four series of samples.

TANNINS

Rhubarb contains a small percentage of astringent substances, or tannins, and it is probable that these are partly responsible for its characteristic flavor. It is not clear just what factors caused the variations of the tannin content of the samples shown in Table 1.

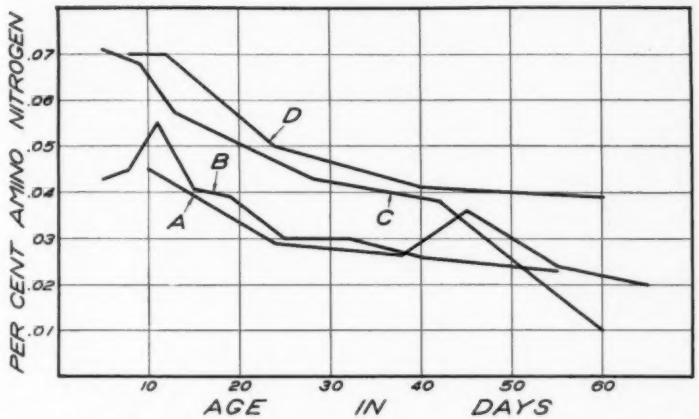

FIGURE 6.—Changes in amino nitrogen content of rhubarb petioles with increase in age: A, Series collected April 21 to June 15; B, series collected June 5; C, series collected July 6; D, series collected October 17

COOKING TESTS

MATERIAL AND METHODS

Rhubarb is commonly used early in the spring, but cooking tests indicate that it may be used at any time throughout the season. In view of the variations in composition as shown by the chemical analyses, some difference in quality from time to time should be expected. The material may be used at any age up to about 30 days when development occurs in the very hottest part of the season, or up to 50 days when development occurs in the cooler part of the season. It becomes somewhat stringy at a much earlier stage in the middle of the summer than in the early spring.

From the standpoint of yield it is not advantageous to harvest when the petioles are only 3 to 4 inches long. The present practice of picking the leaves when the petioles have almost reached their maximum length seems to be about the best procedure. Rhubarb has very nearly maximum moisture content at this stage, yet the difference from earlier stages is not great and may not be very important.

In making the cooking tests some samples were taken in which the petioles were 10 days old and 3 to 4 inches long and, for comparison, other samples were taken that were 10, 18, 30, and 40 days older than the first. The petioles were cut into pieces one-half inch long, placed in a kettle with a small amount of cold water, and allowed to boil for 10 minutes. Then 30 per cent by weight of sugar was added and the material was stirred until the sugar was dissolved. In these tests the very young samples were considered superior to all others. They seemed slightly milder and of better consistency than any of the older ones. There seemed to be very little difference between samples that were taken 18 days and 30 days after the first samples, indicating that the quality remains nearly constant for a considerable time after the petiole ceases its rapid rate of growth. The older samples seemed somewhat more fibrous and less desirable than those taken 18 days

after the first samples. The increase in alcohol-insoluble residue shown by the analytical results indicates that the fiber increases; this is in accordance with the cooking tests.

PECTIN CONTENT

When cooked, rhubarb softens and becomes a viscous, semiliquid, colloidal mass. When tested for starch the result was negative. When strained through cheesecloth the liquid gave a voluminous precipitate with alcohol. A precipitate was also formed with calcium hydroxide. When treated with ammonia and then neutralized, an almost insoluble precipitate was formed. When the very young sample was ground and the juice pressed out without heating, a precipitate was formed with alcohol; but the juice of the older petioles gave very little precipitate when so treated. These tests indicate that there is considerable material of the pectin group in the petiole of the rhubarb plant. A large part seems to be protopectin; pectin and pectic acid are probably present also. While pectin was not determined quantitatively, the difference between the precipitates with alcohol indicated that the protopectin decreased greatly as the petiole became older.

ADJUSTMENT OF THE SUGAR-ACID RATIO AND THE CONSISTENCY

The low sugar content and the high acid content of rhubarb make it necessary to add sugar in order to obtain an agreeable taste. The acidity is sometimes so high that the material is improved by soaking in warm water for a short time or in cold water for a long time. For the same reason, partial neutralization is sometimes recommended; a milder product is thus obtained, which will require less sugar to sweeten properly. The acid content, which has been shown to vary considerably with the season, will influence the quantity of sugar that must be added. In these tests, the addition of 30 to 40 per cent of sugar to material having an acid content of 1 per cent made a sauce agreeable to the writers. Of course, individuals vary greatly in their preference as to sweetness. However, the writers concluded that a sugar ratio of about 1:3 is agreeable to most individuals.

Despite the pectin content the moisture content of rhubarb is so high that in making pies it is usually found desirable to add flour or cornstarch to give the material the proper consistency.

CANNING TESTS

In 1926 and 1927 canning tests were made with rhubarb purchased in the market at Washington, D. C. In 1928 the rhubarb used was grown on the Arlington Experiment Farm, as described earlier in this paper. The age of the material in 1926 and 1927 was not known, but it appeared to be in prime condition for table use. In 1928 the rhubarb collected was 20 to 30 days old. The material was brought to the laboratory, trimmed, washed, cut into pieces one-half inch long, and packed in both tin and glass containers as follows:

(1) The pieces were packed tightly in cans or jars, and water was added to fill the interspaces; the containers were then exhausted three minutes in steam at 100° C. and sealed.

(2) The treatment was the same as in 1 except that 60 per cent sirup was added instead of water.

(3) The treatment was the same as in 1 except that no exhaust was given.

(4) The treatment was the same as in 1, except that 60 per cent sirup was added instead of water and no exhaust was given.
(5) The pieces were steamed for five minutes at 100° C. and while hot were pressed tightly into the cans without the addition of either sugar or water.
(6) The treatment was the same as in 5, except that 40 per cent by weight of sugar was added to the hot material and stirred until dissolved.

Some of the rhubarb receiving each treatment was packed in weighed No. 2 cans of three types, namely, plain tin, single-enamel coke-plate, and reenameled charcoal-plate cans. Additional lots of rhubarb receiving treatments 1 and 3 were packed in No. 3 plain tin and single-enamel coke-plate cans. Additional lots receiving treatments 1, 5, and 6 were packed in quart glass jars.

The cans were processed at 100° C. for 15 minutes and then stored for one year. The tin cans were weighed before they were packed with the material and again when they were opened and examined. Large quantities of yellowish crystals or incrustations that had formed in the can during storage frequently adhered tenaciously. This made the method inaccurate for measuring the total amount of corrosion. It was obvious, however, from the differences in the weight of the cans at the beginning and at the end of the test, that unusually large amounts of tin and iron were dissolved from the cans by the material packed in them.

When examined one year after storage the quality of the rhubarb in the glass jars was very nearly equal to that of rhubarb prepared immediately from fresh material.

All the plain tin cans processed without exhaust (treatments 3 and 4) were perceptibly swelled; some were flippers (slightly swelled), and others were swelled tightly as a result of hydrogen formation. The gas had no foul odor and burned violently when ignited, indicating that it was hydrogen. The cans were severely corroded and frequently weighed 0.5 to 1 g less than when the material was placed in them. The tin coating seemed to have been entirely removed from the iron, which appeared dark in color and in many cases was partly covered with adhering crystals or a yellowish incrustation that could not be readily removed by washing. The rhubarb appeared nearly normal, except that it was light brown in color and dull in appearance as compared with that canned in glass, and had a rather disagreeable, metallic taste. It was on the whole an unsatisfactory product. None of the cans were perforated.

The plain tin cans which were sealed hot (treatments 1, 2, 5, and 6) were frequently slightly swelled, but in many cases were entirely normal in appearance. The tin seemed to have been entirely removed from the interior of the can and it appeared almost as severely corroded as the unexhausted cans. None of the cans had become perforated. The contents of the cans, in every way similar to those of the unexhausted cans, were still distinctly acid, having a pH value of 3.52 to 4.0 (0.0001 to 0.0003 N). Since the iron was apparently exposed, the corrosion might be expected to proceed at a rapid rate; however, it was apparent that the action at the end of one year was very much slowed down if not entirely stopped. This was believed to be due to the formation of protective films or incrustations on the surface of the iron. Yellowish coatings, which probably consisted of crystalline or colloidal iron oxalate, were frequently observed.

All the single-enamel cans showed less gas formation than the plain tin cans and were frequently normal in appearance. They were severely corroded and in every can considerable areas of the enamel were so completely loosened that they flaked away when the contents of the can were emptied. From the areas on which the enamel had loosened it appeared that the tin was also completely removed. It seemed that the tin was dissolved away or oxidized underneath the enamel, which promptly fell off when the can was emptied. Frequently large areas of the iron appeared exposed.

The principal difference between the exhausted and nonexhausted enameled cans was in the number of swelled cans. The difference in the intensity of the corrosive action was not very marked.

The reenameled cans were much less corroded than the single-enamel cans. However, they had many small areas from which the enamel and the tin had been removed. There were no tightly swelled cans, and only a few flippers in the unexhausted lots. The material in the reenameled cans was of fair quality but distinctly less pleasing than that canned in glass. It had a slightly bitter taste, probably due to some reaction with the enamel or to the presence of iron or tin salts of the organic acids present.

The cans treated by the addition of a sugar sirup instead of water were slightly less corroded, but the differences were not very pronounced. The yellowish coating previously mentioned was sometimes quite apparent in these lots.

The cans packed without the addition of water (treatments 5 and 6) were similar to those from the corresponding lots with water added. The corrosion was more intense but not as great as would have been expected as a consequence of the larger quantity of material in these cans.

The results differed somewhat in the three years. The material purchased in the market at Washington, D. C., seemed to act upon the cans more severely than did the variety grown on the Arlington Experiment Farm.

The question arises as to what constituents are responsible for this severe corrosive action on the can. The results in general point very definitely to the presence of two constituents: (1) Oxalic acid or its salts, accompanied by high total acidity due chiefly to malic acid, and (2) nitrates.

Clough and Clark (*8*) conclude that corrosion is due to the oxygen content of the porous stems of the rhubarb. Joslyn (*17*) states that the corrosive action is due to the oxygen content and not to the high acidity. From certain tests made by Kohman (*18*), he concludes that the substance responsible "is not oxygen but a substance that behaves like oxygen." De Fouw (*14*) states that nitrates are the only salts that appreciably affect the rate of corrosion of tin by organic acids. Culpepper and Moon (*11*) have shown that the corrosion of tin cans filled with solutions of organic acids is tremendously increased by the presence of nitrates. Under certain conditions the action occurs without the formation of appreciable amounts of gas. Serger (*29*) finds that in canned meats nitrates may cause corrosion of the tin container. Culpepper and Moon (*13*) have shown that oxalic acid is much more corrosive than other organic acids. Although very little free oxalic acid is present in the petioles, it occurs as the acid

potassium salt. Whether the potassium salt would corrode the can in a similar manner is not known. Serger (*29*) attributes the corrosive action of rhubarb to its oxalic acid content. In the tests here reported the formation of yellowish crystals and a yellowish coating suggests that iron oxalate is one of the products formed. The content of oxalic acid or its salts in rhubarb petioles has been shown by various workers to vary from 0.1 to 0.7 per cent. Culpepper and Moon (*12*) estimated that there was 6 per cent of air by volume in one sample of rhubarb. The gas content is less than this in the young material and greater than this in the old material. Even in the unexhausted cans this would not be sufficient to account for the amount of corrosion that is apparent.

The analytical results of the present investigation indicate that there is at times as high as 0.1 per cent of nitrate nitrogen in the material. A No. 3 can containing 530 g of rhubarb would contain 0.53 g of nitrate nitrogen, or 3.7 g of potassium nitrate. In cases where there is 0.03 per cent of nitrate nitrogen, which is frequently the case at the stage at which rhubarb is canned, there would be 1.1 g of potassium nitrate. This amount is more than ample to account for the action of the material on the can, especially an acid material like rhubarb. Culpepper and Moon (*13*) found that oxalic acid in a concentration of 0.5 per cent caused swelling in one lot of plain tin cans. Therefore it would be expected that if oxalic acid alone were responsible the cans would become tightly swelled. In the tests of Kohman (*18*) the gas formed was not sufficient to swell the can. In the present tests the cans were seldom swelled. The work of Clough and Clark (*8*) and Kohman (*18*) shows that the substance responsible for the swelling is highly soluble in water, which is true of most salts of nitric acid. The behavior in general indicates that nitrate nitrogen, occurring as a constituent in the material, is an important cause of corrosion in canned rhubarb.

The analytical results have shown that there is considerable variation in the nitrate content of rhubarb at different ages and in different seasons. Since soils under different cultural practices may contain various amounts of nitrate nitrogen and since the amount in the soil affects the amount in the plant, one must conclude that the nitrate content of the material used by different workers is subject to wide variation. Inasmuch as the oxalic acid also varies it seems reasonable to suppose that these variations may account for some of the differences in the results of canning tests by different workers.

SUMMARY

A study has been made of the chemical changes that occur in the petioles of rhubarb with increasing age and changing seasonal conditions. It has been shown that these changes influence the cooking and canning quality of the material.

It was found that the total solids did not vary greatly with age. They were high in the very young petioles, decreased for several days when the petioles were elongating most rapidly, increased slightly during the period of maximum photosynthetic activity, and finally decreased somewhat in the very old material. Marked differences were noted in the samples taken at different seasons. The series

taken in July and October were higher in solids than the series taken in April, May, and June.

The titratable acidity did not vary greatly with age until the petioles were very old, when a marked decrease was noted. There was a very marked difference in the acidity of samples taken at different periods of the season. Acidity was high in the series taken in July and October and low in the series taken in April, May, and June.

Rhubarb is very low in sugars and acid-hydrolyzable polysaccharides and variations in the amounts of these constituents are not particularly significant.

The total nitrogen was highest in the young material and decreased somewhat with age. The amino nitrogen likewise decreased with age. The nitrate nitrogen increased greatly as the age of the material increased. These variations were influenced considerably by seasonal conditions.

Qualitative tests indicate that there are present in the rhubarb petiole pectinlike substances that have considerable influence upon the cooking quality of the material. These substances seem to be largely protopectin.

The low carbohydrate content, the high acid content, and the high moisture content are responsible for many of the characteristics of the material as a food product and explain much of its behavior in cooking.

Rhubarb when packed in plain tin cans had a very severe corrosive action upon the metals of the container. The presence of the resulting metallic compounds had a very pronounced detrimental effect upon the taste and appearance of the material. A high-grade reenameled tin can greatly lessened the amount of corrosion and caused a corresponding improvement in the flavor of the material.

Probably one very important factor in the corrosion is the presence of nitrates. Oxalic acid, accompanied by a high malic acid content, is also thought to be of much importance. The variation due to the age of the material and the seasonal conditions in the amount of nitrates present suggests that differences in the nitrate content may be the cause of the differences in the results obtained by different workers.

The results of the present investigation indicate that rhubarb may be used either for cooking or canning at any time during the growing season if care is taken to select petioles at the proper stage of maturity.

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PROGRESSIVE CHANGES IN THE CUTICLE OF APPLES DURING GROWTH AND STORAGE[1]

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INTRODUCTION

In a previous paper (*10*)[3] on the surface covering of apples the work was confined largely to a study of the progressive changes in the waxlike constituents of apples grown on the Arlington Experiment Farm, Rosslyn, Va. It seemed desirable to extend the study to varieties of apples grown in two dissimilar apple regions, namely, the Wenatchee Valley, Wash., and the Finger Lake district of New York, and to include also quantitative cutin determinations. The results of this work are embodied in the present paper.

EXPERIMENTAL MATERIAL

The fruit used in this investigation was of the same lot as that described elsewhere (*11*). Samples of fruit were collected about 55 days after the trees had bloomed and also about the time of picking maturity. A portion of the fruit collected about the time of picking maturity was placed in storage for subsequent work. These samples are referred to, respectively, as early, mature, and storage. Fruit representing the early and the mature stages was sampled at laboratories in the vicinity of the orchards from which it was collected. Material representing the mature and the storage stages of Washington State fruit was picked about a week or 10 days prior to commercial harvesting. The fruit to be stored was packed in boxes with untreated wrappers, shipped under initial ice from Peshastin, Wash., to Philadelphia, and then by express to Washington, D. C. The corresponding samples of New York fruit were picked at commercial harvesting time, packed in lined bushel baskets, and shipped by express to Washington, D. C. Both lots of fruit were placed in the same cold-storage room and kept at 32° F. until removed for analysis.

METHODS OF ANALYSIS

PREPARATION OF SAMPLES AND DETERMINATION OF ETHER-SOLUBLE CONSTITUENTS

The method of sampling and preparation was the same as that described previously (*10*), which briefly is as follows: From the shady side of 75 apples, disks were removed with the aid of a standardized cork borer and immersed for 24 hours in dilute hydrochloric acid. The cuticle was separated from most of the flesh by means of tweezers,

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[3] Reference is made by number (italic) to Literature Cited, p. 412.

washed with distilled water, and dried over sulphuric acid. The total ether extract, ursolic acid, and oily fraction were determined according to the methods which have been described in detail. In the previous paper it was stated that the oily fraction consisted mainly of the hydrocarbon triacontane together with the alcohol heptacosanol and small quantities of plastid pigments and other impurities. It has since been shown by Chibnall and his coworkers (*1*) as well as by the writers (*9*) that the hydrocarbon is *n*-nonacosane and the alcohol 10-nonacosanol.

DETERMINATION OF CUTIN

After the removal of the ether and alcohol soluble constituents from the crude cuticle disks, the residue consisted primarily of cutin [4] admixed with relatively small amounts of impurities derived from the cellular walls and cell contents of adhering epidermal and subepidermal tissues. The presence of such impurities is evidenced from the fact that Rome Beauty cuticle extracted with ether and alcohol and air-dried gave on analysis the following results: Nitrogen, 0.63 per cent (equivalent to 3.94 per cent crude protein); pentosans (Kröber's method), 5.80 per cent; and cellulose, 3.87 per cent. Pectin was present but not quantitatively determined. The presence of lignin could not be shown by the microchemical phloroglucinol-hydrochloric acid test. The quantitative determination of cutin in the presence of these impurities has, in the experience of the writers, been found practically impossible. Even the removal of the impurities in order to determine the cutin residually presented a difficult problem.

König and his coworkers (*4*, *5*), in a study of the chemical components of cellular membranes, particularly the crude-fiber fraction, removed the soluble impurities by treatment of the plant tissues with 2 per cent sulphuric acid in glycerin. After this treatment the residual crude fiber, which consisted of lignin, cellulose, and cutin, was further treated with ammoniacal hydrogen peroxide to oxidize the lignin, and with cuprammonium solution to remove the cellulose. The residue was considered to be cutin. Another method used by König (*6*) consisted in the removal of the cellulose by treatment with 72 per cent sulphuric acid, which was supposed to leave the lignin and cutin unattacked. The lignin in the insoluble portion was oxidized with ammoniacal hydrogen peroxide and the residue weighed as cutin.

As the above-mentioned methods were not considered adaptable in their entirety to the material at hand, a considerable number of preliminary experiments were made in an attempt to improve them. It was found that treatment with hot dilute acid and alkali, although not drastic enough to affect the cutin, materially reduced the noncellulosic impurities. For example, Rome Beauty cuticle, previously

[4] A number of investigators have shown that the apple has a firm, thick cuticle which is structureless and covers the epidermis as a continuous layer except for lenticular openings. If the cuticle is separated from adjacent tissues and then extracted with ether, it may be separated into two main portions, the ether-soluble fraction or waxlike coating consisting of a mixture of normal paraffin hydrocarbons, primary and secondary alcohols, and ursolic acid, and the ether-insoluble portion, which is recognized by many and often referred to as cutin. Knowledge of the chemical nature of the cutin of apples is rather meager, although certain analogies in composition may be drawn from the investigations of Fremy (*2*), Fremy and Urbain (*3*), Van Wisselingh (*13*), Sutthoff (*12*), Lee (*7*), and Legg and Wheeler (*8*), who made studies on cutin from other sources. As a result of this work it may be concluded that cutin consists of an esterlike substance which on saponification yields a number of incompletely identified solid and semiliquid acids.

extracted with ether and alcohol, lost 16.34 per cent impurities when refluxed 30 minutes with 0.2 per cent aqueous potash, followed by 30 minutes, refluxing with 1.25 per cent sulphuric acid. Comparable samples, treated in the same manner, except for allowing the samples to stand overnight in contact with cold aqueous alkali, lost 13.90 per cent in weight. In the latter case, however, the crude cellulose content was slightly higher, which indicated that a portion of the unattacked impurities remained with the cellulose. That nearly all the impurities were removed in the hot alkali and acid treatments was shown by the fact that the oven-dried cutin-cellulose residue contained but 0.1 per cent ash, 0.45 per cent nitrogen, and 1.53 per cent pentosans. Moreover, some of these impurities were associated with the cellulose, as was subsequently determined.

After removal of most of the impurities by hot alkali and acid digestion, treatment of the cutin-cellulose portion with 72 per cent sulphuric acid to remove the cellulose gave a slimy, partly carbonized residue amounting to 47.54 per cent. According to König this residue should represent cutin and lignin, although it is apparent that the percentage is much too low because of the partial decomposition of the cutin by sulphuric acid. Similar cutin-cellulose samples treated with cuprammonium reagent to remove the cellulose gave cutin residues equivalent to 95.75 and 96.23 per cent. In view of the wide divergence in the results of the two methods, neither could be accepted. From the standpoint of accuracy, the cuprammonium method is undoubtedly to be preferred, but it is tedious and lengthy in operation.

It has been known for a long time (*2*) that cutin is saponified completely with alcoholic potash, and it was thought that the cutin might be quantitatively separated from the cellulose by this means. It was found that saponification for three hours with 3 per cent alcoholic potash gave results quite comparable with the cuprammonium method. For example, triplicate determinations on samples of Rome Beauty cutin-cellulose residues comparable with those used previously gave 95.3, 95.6, and 95.2 per cent cutin by difference. The crude cellulose residue obtained after saponification of the cutin with 3 per cent alcoholic potash gave on analysis 0.1 per cent ash, 0.9 per cent nitrogen (equivalent to 5.62 per cent protein), and 5.16 per cent pentosans. Incidentally the results of the above experiments seem to indicate that the cellulose exists chemically uncombined with the cutin rather than as a definite chemical entity, termed cutocellulose by earlier workers.

Based on preliminary work, part of which is described above, the following empirical method for the determination of the cutin present in apple cuticle was devised and applied throughout the series of experiments herein reported. After extraction of the cuticular disks with ether and alcohol each sample was transferred to a 500-ml Erlenmeyer flask fitted with a ground-glass-joint condenser, refluxed for 30 minutes with 100 ml of 0.2 per cent aqueous potash, filtered through a 35-ml No. 3 Jena-glass crucible with fritted bottom, thoroughly washed with boiling water, and finally dried enough to permit easy removal of the residue from the crucible. The residue was returned to the original flask, refluxed for 30 minutes with 1.25 per cent sulphuric acid, filtered through the same crucible as before, and thoroughly washed with hot water and alcohol. The alkali and

acid treated material was then dried at 110° C. and cooled in a desiccator over sulphuric acid. The weighed cutin-cellulose residue was removed from the crucible and saponified by refluxing for three hours with 100 ml of 3 per cent alcoholic potash. The insoluble cellulosic material was separated by filtration from the hot liquid and washed with hot alcohol and water, after which it was dried to constant weight at 110° C. It was then cooled and weighed. The difference in the two weighings represents the weight of cutin saponified.

TABLE 1.—*Progressive changes in the ether-soluble surface constituents and cutin from the shady side of apples from Washington during maturation and in storage*

Variety and stage of growth	Date of sampling	Age from blooming	Minimum and maximum diameters of apples used	Total area of 75 disks	Constituents per 100,000 mm² of surface area				Oily or petroleum ether soluble fraction in total ether extract
					Ursolic acid	Oily or petroleum ether soluble fraction	Total ether extract	Cutin	
FALL VARIETIES									
McIntosh:		*Days*	*Mm*	*Mm²*	*Mg*	*Mg*	*Mg*	*Mg*	*Per cent*
Early	June 28, 1929	55	36–48	29, 218	398. 7	137. 9	536. 6	663. 5	25. 7
Mature	Sept. 4, 1929	123	64–77	27, 901	463. 8	206. 4	670. 2	803. 2	30. 8
Storage	Jan. 3, 1930	244	66–76	27, 911	515. 6	289. 1	804. 7	950. 0	35. 9
Wagener:									
Early	June 30, 1929	57	43–52	28, 887	379. 1	199. 7	578. 8	822. 6	34. 5
Mature	Sept. 17, 1929	136	70–86	27, 780	382. 3	294. 5	676. 8	924. 0	43. 5
Storage	Jan. 3, 1930	244	69–88	27, 762	422. 1	300. 8	722. 9	1, 085. 6	41. 6
Delicious:									
Early [a]	June 27, 1929	54	35–44	28, 664	497. 2	217. 0	714. 2	931. 2	30. 4
Mature	Sept. 25, 1929	144	63–81	27, 902	549. 8	296. 4	846. 2	1, 217. 5	35. 0
Storage	Mar. 5, 1930	305	66–81	27, 884	612. 1	529. 7	1, 141. 8	1, 257. 2	46. 4
Grimes Golden:									
Early [a]	June 28, 1929	55	33–41	29, 503	394. 8	213. 2	608. 0	907. 9	35. 1
Mature	Sept. 4, 1929	123	63–77	27, 898	445. 8	386. 7	832. 5	1, 447. 2	46. 5
Storage	Jan. 17, 1930	258	63–79	27, 541	576. 6	513. 8	1, 090. 4	1, 632. 8	47. 1
LATE VARIETIES									
Rome Beauty:									
Early	June 26, 1929	53	33–42	30, 077	377. 1	162. 9	540. 0	687. 6	30. 2
Mature	Oct. 3, 1929	152	68–88	27, 777	536. 4	328. 0	864. 4	1, 107. 9	37. 9
Storage	Feb. 12, 1930	284	69–89	27, 781	623. 5	381. 6	1, 005. 1	1, 206. 4	38. 0
Ben Davis:									
Early	June 28, 1929	55	35–45	29, 750	440. 9	196. 3	637. 2	841. 2	30. 8
Mature	Sept. 27, 1929	146	63–84	27, 846	494. 5	302. 3	796. 8	858. 2	37. 9
Storage	Feb. 18, 1930	290	67–82	27, 842	623. 6	367. 4	991. 0	938. 8	37. 1
Arkansas Black:									
Early [a]	June 29, 1929	56	35–44	29, 429	500. 9	192. 6	693. 5	972. 8	27. 8
Mature	Oct. 8, 1929	157	67–77	27, 876	681. 5	449. 8	1, 131. 3	1, 295. 3	39. 8
Storage	Mar. 5, 1930	305	70–85	27, 796	836. 2	554. 8	1, 391. 0	1, 346. 6	39. 9
York Imperial:									
Early	June 29, 1929	56	38–42	29, 685	363. 9	189. 3	553. 2	892. 1	34. 2
Mature	Oct. 4, 1929	153	70–80	27, 824	547. 0	370. 2	917. 2	1, 181. 7	40. 4
Storage	Feb. 25, 1930	297	67–77	27, 854	548. 2	412. 8	961. 0	1, 185. 2	43. 0
Stayman Winesap:									
Early	June 26, 1929	53	35–42	29, 776	497. 7	193. 4	691. 1	1, 041. 6	28. 0
Mature	Sept. 28, 1929	152	66–87	27, 813	535. 6	381. 1	916. 7	1, 416. 1	41. 6
Storage	Mar. 13, 1930	313	69–81	27, 805	601. 2	446. 3	1, 047. 5	1, 481. 8	42. 6
Winesap:									
Early	June 27, 1929	54	33–38	30, 342	491. 1	175. 3	666. 4	923. 9	26. 3
Mature	Oct. 7, 1929	156	63–75	27, 976	531. 1	391. 7	922. 8	1, 473. 2	42. 4
Storage	Mar. 20, 1930	320	64–73	27, 970	599. 2	490. 5	1, 089. 7	1, 467. 9	45. 0
Northern Spy:									
Mature	Sept. 5, 1929	124	63–80	28, 254	349. 6	278. 2	627. 8	972. 5	44. 3
Storage	Feb. 12, 1930	284	67–81	27, 854	409. 3	348. 2	757. 5	1, 119. 5	46. 0
Esopus Spitzenburg:									
Early	June 27, 1929	54	36–43	29, 629	415. 1	176. 9	592. 0	898. 1	29. 9
Mature	Sept. 26, 1929	145	66–78	27, 861	595. 1	479. 8	1, 074. 9	1, 415. 5	44. 6
Storage	Jan. 17, 1930	258	67–77	27, 877	666. 5	505. 7	1, 172. 2	1, 370. 8	43. 1
Yellow Newtown:									
Early [a]	June 30, 1929	57	38–47	29, 350	367. 3	194. 9	562. 2	920. 2	34. 7
Mature [a]	Oct. 4, 1929	153	66–84	27, 845	401. 1	379. 6	780. 7	1, 047. 1	48. 6
Storage	Apr. 17, 1930	348	66–83	27, 854	456. 7	452. 3	909. 0	1, 055. 8	49. 8

[a] Mixed sample; no difference discernible between shady and sunny sides of same fruit.

TABLE 2.—*Progressive changes in the ether-soluble surface constituents and cutin from the shady side of apples from New York during maturation and in storage*

Variety and stage of growth	Date of sampling	Age from blooming	Minimum and maximum diameters of apples used	Total area of 75 disks	Constituents per 100,000 mm² of surface area				Oily or petroleum ether soluble fraction in total ether extract
					Ursolic acid	Oily or petroleum ether soluble fraction	Total ether extract	Cutin	
FALL VARIETIES									
McIntosh:		*Days*	*Mm*	*Mm²*	*Mg*	*Mg*	*Mg*	*Mg*	*Per cent*
Early	July 6, 1929	52	31–41	29,386	337.9	143.3	481.2	596.5	29.8
Mature	Sept. 24, 1929	132	56–68	28,129	493.1	251.7	744.8	873.1	33.8
Storage	Jan. 3, 1930	233	57–68	28,129	578.8	289.7	868.5	980.3	33.4
Wagener:									
Early	July 6, 1929	52	31–38	29,993	361.4	168.4	529.8	679.8	31.8
Mature	Oct. 10, 1929	148	53–70	28,227	503.1	353.6	856.7	799.3	41.3
Storage	Jan. 3, 1930	233	58–70	28,100	565.5	381.2	946.7	1,076.8	40.3
Delicious:									
Early	July 8, 1929	52	30–38	30,820	410.7	226.7	637.4	738.6	35.6
Mature	Oct. 14, 1929	150	49–61	28,400	617.6	491.2	1,108.8	1,011.9	44.3
Storage	Mar. 5, 1930	292	51–67	28,223	637.4	517.3	1,154.7	1,121.8	44.8
Grimes Golden:									
Early	July 10, 1929	55	26–36	32,286	397.0	232.6	629.6	871.2	36.9
Mature	Sept. 30, 1929	137	50–59	28,472	466.7	455.9	922.6	1,426.6	49.4
Storage	Dec. 17, 1929	215	50–61	28,450	599.3	477.0	1,076.3	1,663.0	44.2
LATE VARIETIES									
Rome Beauty:									
Early	July 12, 1929	53	30–40	30,667	375.0	191.1	566.1	733.1	33.8
Mature	Oct. 18, 1929	151	51–67	28,261	531.8	353.1	884.9	1,091.1	39.9
Storage	Feb. 12, 1930	268	62–76	27,941	579.8	407.6	987.4	----------	41.3
Ben Davis:									
Early	July 10, 1929	54	28–40	31,016	402.7	215.7	618.4	804.1	34.9
Mature	Oct. 17, 1929	153	53–67	28,210	759.7	404.1	1,163.8	1,166.0	34.7
Storage	Feb. 20, 1930	279	57–70	28,098	814.6	544.2	1,358.8	1,463.5	40.1
Arkansas:									
Early	July 16, 1929	58	39–49	29,180	510.3	208.3	718.6	1,013.7	29.0
Mature	Oct. 8, 1929	142	65–85	27,866	573.2	303.6	876.8	1,254.0	34.6
Storage	Jan. 21, 1930	247	63–87	27,862	672.9	365.0	1,037.9	1,492.4	35.2
Baldwin:									
Early	July 8, 1929	53	34–44	29,609	410.6	189.1	599.7	864.2	31.5
Mature	Oct. 14, 1929	151	57–75	28,075	560.6	354.8	915.4	1,072.2	38.8
Storage	Feb. 18, 1930	278	58–76	28,010	646.9	489.8	1,136.7	----------	43.1
Stayman Winesap:									
Early	July 16, 1929	59	33–42	30,203	526.4	226.2	752.6	1,036.7	30.1
Mature	Oct. 8, 1929	143	51–72	28,148	647.4	365.6	1,013.0	1,386.4	36.1
Storage	Mar. 13, 1930	299	55–73	28,137	801.4	456.3	1,257.7	----------	36.3
Winesap:									
Early	July 16, 1929	57	29–38	31,408	495.4	228.3	723.7	965.1	31.5
Mature	Oct. 8, 1929	141	54–62	28,281	610.3	329.9	940.2	1,247.5	35.1
Storage	Mar. 20, 1930	304	50–62	28,340	713.7	449.8	1,163.5	1,589.2	38.7
Northern Spy:									
Early	July 12, 1929	52	32–50	29,704	353.8	190.2	544.0	637.5	35.0
Mature	Oct. 18, 1929	150	53–76	28,138	500.4	340.5	840.9	957.8	40.5
Storage	Feb. 12, 1930	267	54–76	27,723	560.5	373.7	934.2	1,131.2	40.0
Esopus Spitzenburg:									
Early	July 9, 1929	53	29–37	31,097	417.8	218.0	635.8	888.2	34.3
Mature	Sept. 30, 1929	136	53–67	28,243	558.4	451.8	1,010.2	1,257.8	44.7
Storage	Dec. 17, 1929	214	50–64	28,325	685.2	462.8	1,148.0	1,407.4	40.3
Yellow Newtown:									
Early	July 12, 1929	53	29–38	31,101	345.9	216.7	562.6	870.0	38.5
Mature	Oct. 17, 1929	150	47–57	28,591	508.3	354.7	863.0	893.7	41.1
Storage	Mar. 5, 1930	289	48–56	28,551	491.7	389.4	881.1	1,007.4	44.2
Rhode Island Greening:									
Early	July 9, 1929	53	34–41	29,184	354.2	187.4	541.6	739.3	34.6
Mature	Sept. 25, 1929	131	56–70	28,067	476.4	327.1	803.5	1,104.5	40.7
Storage	Jan. 21, 1930	249	59–72	28,043	557.7	369.4	927.1	1,308.6	39.8

PROGRESSIVE CHANGES IN WAXLIKE CONSTITUENTS AND CUTIN DURING GROWTH AND STORAGE

Data reported in Tables 1 and 2 represent the early, mature, and storage stages for the shady side of fruit grown in Washington State and in New York. In the previous paper (*10*) it was shown that the quantities of ursolic acid, oily fraction, and total ether extract present

at maturity and at the end of the storage period were invariably greater than those found in the early stage of growth, and that in general the constituents increased from the time of picking to the end of the storage period. The results reported in the present paper confirm the previous conclusions. It was also shown that while the quantities of both ursolic acid and the oily fraction became larger with increasing maturity, the oily fraction increased at a faster rate than did the ursolic acid; hence with advancing maturity there was in general a progressive increase in the percentage of the oily fraction in the total ether extract. This condition with one exception (Ben Davis from New York) is also found to be true in the present study.

In connection with the cutin determinations reported in the same tables it is found that the same general conclusions can be drawn as in the case of the waxlike constituents. For example, the quantities of cutin were found to be greater at maturity and at the end of the storage period than in the early stage. With two exceptions (Winesap and Esopus Spitzenburg from Washington), the quantities of cutin found at the end of the storage period were greater than at picking maturity. It is not clear why in many cases there should be unusually large differences in the quantities of cutin found at the end of the storage period as compared with those found at picking maturity, since it is difficult to conceive how cutin would continue to be formed after the fruit has been picked. It is easy to understand how the oily fraction and the ursolic acid might increase during storage, since it is entirely possible that the constituents representing the oily fraction may diffuse outward from the interior of the fruit and that ursolic acid may be formed as a result of oxidation or condensation of cell-wall progenitors. Of course the latter hypothesis may also afford an explanation of the observed cutin increases during the storage period.

COMPARISON BETWEEN FRUIT GROWN IN WASHINGTON AND NEW YORK

At the mature stage the mean values, in milligrams per 100,000 mm^2 of surface area, for only those varieties grown in both Washington and New York, are, respectively: Ursolic acid, 480.5 and 563.3; oily fraction, 338.6 and 377.5; total ether extract, 819.1 and 940.8; cutin, 1,152.9 and 1,095.6; percentage of oily fraction in total ether extract, 41.3 and 40.1. At the storage stage the corresponding values are: Ursolic acid, 555.1 and 638.9; oily fraction, 420.5 and 431.7; total ether extract, 975.6 and 1,070.6; cutin, 1,233.3 and 1,271.2; percentage of oily fraction in total ether extract, 43 and 40.3. These figures indicate that the New York apples, when considered as a group, had higher amounts of ursolic acid, oily fraction, and total ether extract at the mature stage and at the end of the storage period than did the Washington apples. On the other hand, the percentage of oily fraction in the total ether extract was higher for both stages in the Washington fruit.

When individual varieties are considered it is found that, with the exception of Esopus Spitzenburg and Rome Beauty, ursolic acid is higher for the New York than for the corresponding Washington fruit. Likewise all New York varieties are higher in oily fraction and total ether extract except Esopus Spitzenburg, Winesap, Stayman Winesap, and Yellow Newtown, which are lower in oily fraction, and Esopus Spitzenburg, which is lower in total ether extract.

It should be borne in mind that variations in total quantities of constituents may be due to (1) differences in the average daily rate of deposition for the same length of growing season, or to (2) differences in length of growing period where the average daily rate of deposition is the same. For example, the average daily rates of deposition, which are given in column 6, Table 3, for the total ether extract representing Yellow Newtown from Washington and New York in 1929 for practically the same period of growth (153 and 150 days), are 5.10 and 5.75 mg, respectively, thereby resulting in a lower total ether extract for Washington fruit than for New York fruit (780.7 and 863 mg). On the other hand, in the case of Grimes Golden from the two localities the average daily rates of deposition are practically the same (6.76 and 6.73 mg.), whereas the lengths of growing season are 123 and 137 days and the quantities of total ether extract 832.5 and 922.6 mg for Washington and New York, respectively.

TABLE 3.—*Comparative data representing the mature stage, shady side, for fall and late varieties of apples from several localities*

Variety, locality, and year	Age from blooming	Constituents per 100,000 mm² of surface area					Oily or petroleum ether soluble fraction in total ether extract
		Ursolic acid	Oily or petroleum ether soluble fraction	Ether extract		Cutin	
				Total	Average daily rate of deposition		
FALL VARIETIES							
McIntosh:	*Days*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Per cent*
Virginia, 1927	149	596.8	284.1	880.9	5.91	----------	32.3
British Columbia, 1927	----------	807.6	420.3	1,227.9	----------	----------	34.2
Washington, 1929	123	463.8	206.4	670.2	5.45	803.2	30.8
New York, 1929	132	493.1	251.7	744.8	5.64	873.1	33.8
Jonathan:							
Virginia, 1926	149	567.3	324.1	891.4	5.98	----------	36.4
Washington, 1927	----------	542.8	299.6	842.4	----------	----------	35.6
Washington, 1929 [a]	151	555.1	352.0	907.1	6.01	1,094.7	38.8
Do.[b]	150	511.1	274.9	786.0	5.24	853.7	35.0
Do.[c]	153	481.0	321.5	802.5	5.24	946.4	40.0
Do.[d]	153	538.1	351.2	889.3	5.81	929.0	39.5
Winter Banana:							
Virginia, 1926	132	525.2	312.9	838.1	6.35	----------	37.3
Virginia, 1927	141	487.5	308.0	795.5	5.64	----------	38.7
Washington, 1927	----------	619.1	443.5	1,062.6	----------	----------	41.7
Delicious:							
Virginia, 1926	160	505.7	398.8	904.5	5.65	----------	44.1
Virginia, 1927	154	747.6	448.7	1,196.3	7.76	----------	37.5
Washington, 1927	----------	437.5	269.1	706.6	----------	----------	38.1
Washington, 1929	144	549.8	296.4	846.2	5.88	1,217.5	35.0
New York, 1929	150	617.6	491.2	1,108.8	7.39	1,011.9	44.3
Wagener:							
Washington, 1929	136	382.3	294.5	676.8	4.98	924.0	43.5
New York, 1929	148	503.1	353.6	856.7	5.79	799.3	41.3
Grimes Golden:							
Virginia, 1926	167	572.6	610.1	1,182.7	7.08	----------	51.6
Virginia, 1927	152	588.9	500.6	1,089.5	7.16	----------	45.9
Washington, 1927	----------	658.2	421.0	1,079.2	----------	----------	39.0
Washington, 1929	123	445.8	386.7	832.5	6.76	1,447.2	46.5
New York, 1919	137	466.7	455.9	922.6	6.73	1,426.6	49.4

[a] Yakima, sprayed with lead arsenate and oil.
[b] Yakima, unsprayed.
[c] Wenatchee, unsprayed.
[d] Wenatchee, sprayed with lead arsenate.

TABLE 3.—*Comparative data representing the mature stage, shady side, for fall and late varieties of apples from several localities*—Continued

Variety, locality, and year	Age from blooming	Constituents per 100,000 mm² of surface area					Oily or petroleum ether soluble fraction in total ether extract
		Ursolic acid	Oily or petroleum ether soluble fraction	Ether extract		Cutin	
				Total	Average daily rate of deposition		
LATE VARIETIES							
Baldwin:	*Days*	*Mg*	*Mg*	*Mg*	*Mg*	*Mg*	*Per cent*
Virginia, 1927	153	579.4	322.6	902.0	5.90		35.8
New York, 1929	151	560.6	354.8	915.4	6.06	1,072.2	38.8
Stayman Winesap:							
Washington, 1929	152	535.6	381.1	916.7	6.03	1,416.1	41.6
New York, 1929	143	647.4	365.6	1,013.0	7.08	1,386.4	36.1
Arkansas Black:							
Virginia, 1926	140	722.3	395.6	1,117.9	7.50		35.4
Virginia, 1927	159	624.5	397.2	1,021.7	6.43		38.9
Washington, 1927		542.5	392.3	934.8			42.0
Washington, 1929	157	681.5	449.8	1,131.3	7.20	1,295.3	39.8
Ben Davis:							
Virginia, 1926	180	502.7	400.2	902.9	5.01		44.3
Washington, 1929	146	494.5	302.3	796.8	5.46	858.2	37.9
New York, 1929	153	759.7	404.1	1,163.8	7.61	1,106.0	34.7
Rome Beauty:							
Virginia, 1926	183	552.8	382.2	935.0	5.11		40.9
Washington, 1929	152	536.4	328.0	864.4	5.69	1,107.9	37.9
New York, 1929	151	531.8	353.1	884.9	5.86	1,091.1	39.9
Winesap:							
Washington, 1929 [a]	150	640.8	360.3	1,001.1	6.67	1,678.2	36.0
Do.[e]	150	542.5	455.3	997.8	6.65	1,506.2	45.6
Do.[f]	156	531.1	391.7	922.8	5.91	1,473.2	42.4
New York, 1929	141	610.3	329.9	940.2	6.67	1,247.5	35.1
Northern Spy:							
Washington, 1929	124	349.6	278.2	627.8	5.06	972.5	44.3
New York, 1929	150	500.4	340.5	840.9	5.61	957.8	40.5
Rhode Island Greening:							
Virginia, 1927	152	463.0	366.5	829.5	5.46		44.2
New York, 1929	131	476.4	327.1	803.5	6.13	1,104.5	40.7
Yellow Newtown:							
Washington, 1929 [g]	158	611.5	527.7	1,139.2	7.21	1,327.1	46.3
Do.[h]	158	534.9	400.7	935.6	5.92	1,005.8	42.8
Do.[i]	153	401.1	379.6	780.7	5.10	1,047.1	48.6
New York, 1929	150	508.3	354.7	863.0	5.75	893.7	41.1
York Imperial:							
Virginia, 1926	187	514.2	470.0	984.2	5.26		47.8
Virginia, 1927	180	558.4	506.4	1,064.8	5.91		47.6
Washington, 1929	153	547.0	370.2	917.2	5.99	1,181.7	40.4
Esopus Spitzenburg:							
Washington, 1927		474.0	451.5	925.5			48.8
Washington, 1929	145	595.1	479.8	1,074.9	7.41	1,415.5	44.6
New York, 1929	136	558.4	451.8	1,010.2	7.43	1,257.8	44.7

[a] Yakima, unsprayed.
[e] Yakima, sprayed with lead arsenate and oil.
[g] Different strain from normal variety.
[h] Different strain from *g* and normal variety.
[i] Normal variety.

It should also be noted that the average daily rate of deposition of the total ether extract for individual varieties is higher for the same varieties grown in New York during 1929 than for those grown in Washington, except for Grimes Golden, in which case the value is practically the same for both localities.

COMPARATIVE DATA REPRESENTING THE MATURE STAGE FOR EACH VARIETY FROM SEVERAL LOCALITIES

In Table 3 a summary is given of data, including some previously reported results, representing quantities of constituents and percentage of oily fraction in the total ether extract found on the shady side of the fruit at picking maturity for all varieties that were collected

from two or more localities. These data make it possible to observe more clearly than hitherto the variations for individual varieties that occur as a result of somewhat different environmental conditions and, to a lesser extent, differences due to variations in length of growing period. The localities and seasons of growth represented by the various samples include the irrigated Wenatchee and Yakima Valleys, an unusually dry summer in the Finger Lake district, and a normal and dry summer at the Arlington farm in Virginia.

Since the environments may be considered as somewhat extreme, the range in values for the ether-soluble constituents for a particular variety in most cases may be considered to include the most probable values for these substances. The table shows that some varieties like Rome Beauty, Baldwin, and Rhode Island Greening show only small variations in the values of the ether-soluble constituents despite considerable variations in growing conditions, while others like Grimes Golden, Delicious, and McIntosh show considerable variations in values.

In column 6, of Table 3, the average daily rates of deposition of total ether extract are given for those varieties for which complete data are available. These values reflect variations due to environment independent of the length of the period of growth and may, therefore, be more characteristic of varieties than is the total quantity of the ether extract.

When the total ether extract and cutin are averaged for the varieties for which data are available, the ratio of total ether extract to cutin is in the proportion of about 44 to 56. In other words, the main outer protective coating of apples is made up of approximately 44 per cent of ether-soluble materials and 56 per cent of ether-insoluble cutin.

SUMMARY AND CONCLUSIONS

Further studies on the progressive changes in the waxlike coating on the surface of the shady side of the apple during growth and storage have been made on varieties grown in two dissimilar apple regions, namely, the Wenatchee Valley, Wash., and the Finger Lake district of New York. A method for the quantitative determination of cutin, present in the cuticle of apples, has been devised and used in the present study.

In confirmation of previous results, obtained from fruit grown on the Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C., it was found with New York and Washington fruit that quantities of ursolic acid, oily fraction, and total ether extract present at maturity and at the end of the storage period were greater than those found in the early stage of growth and that the quantities of the constituents increased from the time of picking to the end of the storage period. It was also found that with advancing maturity there was in general a progressive increase in the percentage of the oily fraction in the total ether extract.

Quantities of cutin were greater at maturity and at the end of the storage period than in the early stage. The proportion of total ether extract to cutin varies considerably in individual varieties, but when these substances representing the mature stage are averaged for the varieties for which complete data are available, the ratio of total ether extract to cutin is about 44 to 56, indicating that the cuticle of apples, which constitutes the main outer protective coating, is com-

posed of approximately 44 per cent of ether-soluble constituents and 56 per cent of ether-insoluble cutin.

In considering comparable apples from each locality as a group, New York fruit in 1929 had higher amounts of ursolic acid, oily fraction, and total ether extract at the mature stage and at the end of the storage period than did Washington apples. Individually the same varieties from the two localities varied somewhat with respect to quantities of constituents.

Data are given showing the quantities of constituents and percentage of oily fraction in the total ether extract at picking maturity for all varieties collected from two or more localities and the average daily rates of deposition of total ether extract for most of the varieties. These values may possibly be characteristic of varieties, since they reflect differences in quantities of total ether extract due to environment and hereditary tendencies independent of the length of growing season.

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INHERITANCE OF BUNT REACTION AND OTHER CHARACTERS IN HOPE WHEAT CROSSES [1]

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INTRODUCTION

Bunt, or stinking smut, caused by *Tilletia tritici* (Bjerk.) Wint. or by *T. levis* (Kuehn), long has been a serious disease of wheat in the Pacific Northwest. In recent years it has become an important problem in Montana. Winter wheat is more severely affected by bunt than is spring wheat, but since the commercial distribution of certain new varieties, particularly Kota, Supreme, and Ceres, bunt has become more prevalent in spring wheat.

A study has been made of bunt reaction and certain other characters in the parents and F_3 hybrid strains of crosses between Hope wheat and three other varieties, namely, Ceres, Marquis, and Hard Federation. The present paper reports the results of this investigation.

REVIEW OF LITERATURE

Inherent differences in the reaction of wheat varieties to bunt were reported by Farrer (*7*)[2] in 1901. Since then varietal reaction has been investigated and reviewed by several workers (*11, 13, 14*). An almost complete range of reaction has been obtained with different varieties, varying from immune and resistant to susceptible, each inherently different but with some overlapping. No immune variety of common spring wheat has been found. In winter-wheat hybrids immunity is inherited as a dominant character in contrast with resistance, which is inherited as a recessive character. Immunity from some forms of bunt, or stinking smut, has been obtained in Martin and Hussar, two winter wheats. In crosses studied by Briggs (*2, 3*) immunity has been found to segregate in simple Mendelian ratios, the result of one or two genetic-factor differences, respectively. In other studies, where different degrees of resistance and susceptibility are involved, the results have not been so definite and indicate that several factors are involved.

Different physiologic forms of bunt have been reported recently by Gaines (*8*) and Rodenhiser (*12*). In some sections new forms have increased the problems of the plant breeder.

MATERIAL AND METHODS

Marquis, the principal commercial variety of hard red spring wheat grown in Montana, has some resistance to bunt, and partly for this reason the losses in spring wheat have been less serious than those in

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[2] Reference is made by number (italic) to Literature Cited, p. 425.

winter wheat. Hope spring wheat has a lower percentage of bunt or a greater resistance than Marquis. This stronger resistance of Hope has been studied in crosses involving the weaker resistance of Marquis and different degrees of susceptibility in Ceres and Hard Federation. Three crosses have been tested with a collection of *Tilletia levis* from Bozeman, Mont.

Since the presence of more than one physiologic form of bunt would complicate the problem, a preliminary study was made of the material used in the present investigation. In 1929 collections of bunt from various localities in Montana were tested individually on the parent wheat varieties at Bozeman. Since no differential reactions were obtained, the collections were assumed to represent only one form.

The parent wheat varieties and the F_3 hybrid progenies were grown at the Montana Agricultural Experiment Station, Bozeman, Mont., in 1929. With one exception, the F_2 material was grown, under bunt-free conditions, at the United States Northern Great Plains Field Station, Mandan, N. Dak., in 1928. The F_2 plants of the Hope × Hard Federation cross were grown in a greenhouse at the Arlington Experiment Farm, Rosslyn, Va. (near Washington, D. C.), in the winter of 1928–29.

The seed of the parent varieties and of the F_2 plants was inoculated with *Tilletia levis* by shaking an excess quantity of spores and the seed wheat in a glass container. The F_3 hybrid and parent plants were grown in rod rows 1 foot apart and were spaced 3 inches apart in each row. The parent checks occupied every tenth row, and the F_3 strains, arranged in the random order of the harvesting of the F_2 plants, occupied the intervening rows.

After harvest, the individual plants in each row were studied for bunt infection. The studies on the Hope × Marquis and Hope × Ceres crosses were made in the field at Bozeman, while those on the Hope × Hard Federation cross were made in the laboratory at Washington, D. C. The latter cross was more closely studied than the others, as each apparently bunt-free plant was threshed separately, and if a trace of bunt was observed during or after threshing, the plant was classified as bunted.

EXPERIMENTAL DATA

INHERITANCE OF REACTION TO BUNT

The parent reaction in percentage of bunted plants and the segregation in the three crosses, Hope × Marquis, Hope × Ceres, and Hope × Hard Federation, as shown by the average of F_3 hybrid strains, are shown in Table 1, and graphically in Figure 1.

The data on the hybrids show a segregation covering almost the complete range of the parents, except that in the Hope × Hard Federation cross there were no F_3 bybrid strains within the limits of the Hope parent. In this cross there is a preponderance of very susceptible strains. The absence of strains having the strong resistance of the Hope parent indicates that in this cross smut reaction is determined by a larger number of genetic factors than in the other crosses. Apparently a sufficient number of strains was not grown to recover the Hope genotype. In the Hope × Ceres cross an intermediate inheritance was indicated, 12 of the 103 strains having a

reaction similar to that of the Hope parent. At the other end of the curve there were 18 strains within the limits of the Ceres parent. These results indicate that at least two genetic factors for bunt reaction are involved in this cross, since on the basis of a 1-factor difference, one-fourth, or about 26 strains, would be expected to fall within the range of each parent.

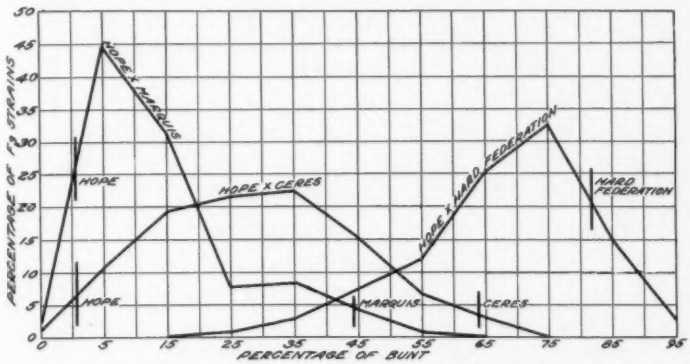

FIGURE 1.—Frequency distribution of the average percentage of bunted plants of F_3 hybrid strains for three wheat crosses, together with the average of the four parents, at Bozeman, Mont., 1929

TABLE 1.—*Percentages of bunted wheat plants in rows of Hope, Marquis, Ceres, and Hard Federation parents and F_3 hybrid strains of crosses between Hope and these other varieties grown at Bozeman, Mont., in 1929*

Percentage of bunted plants	Number of rows of indicated parent				Number of rows of F_3 hybrid strains of indicated cross		
	Hope	Marquis	Ceres	Hard Federation	Hope × Marquis	Hope × Ceres	Hope × Hard Federation
0.0	7				2	1	
2.5	9				19	2	
7.5	2				22	9	
12.5					9	6	
17.5					20	14	
22.5					4	11	1
27.5					3	11	1
32.5					5	16	
37.5		2			3	7	3
42.5		1			2	8	2
47.5		2	1		2	8	6
52.5		1			1	4	5
57.5			2			3	8
62.5			1			2	8
67.5				1		1	19
72.5			1				15
77.5				2			20
82.5							9
87.5			1	2			7
92.5				1			3
97.5							
Total	18	6	6	6	92	103	107
Average [a]	2.1	44.2	64.2	81.7	14.7	29.3	68.5
P. E	±.4	±1.7	±3.9	±2.7	±.9	±1.0	±.9

[a] Average percentage of bunted plants.

In the Hope × Marquis cross there is a preponderance of strains approaching the stronger resistance of the Hope parent. Of the 92 strains, 43 were within the limits of the Hope parent; only 8 strains

were within the limits of the Marquis parent. It is recognized that the number of parent check rows was comparatively small and may not give a true expression of the parental range.

None of these data indicates a simple single-factor difference, or a 1:2:1 segregation, as found by Briggs (*2*) in crosses with the immune variety Martin. In general, the results from the three crosses indicate that the stronger the degree of resistance involved in the hybrids the less complicated the inheritance, with an increasing tendency away from an imperfect dominance of susceptibility to an imperfect dominance of strong resistance.

SEGREGATION OF VARIOUS CHARACTERS IN HOPE × HARD FEDERATION

In addition to data on percentage of bunted plants, other data were obtained on the Hope × Hard Federation cross, including the percentage of bunt, yield per plant, glume color, kernel color, and awnedness. These data are presented in the order given, together with the relation between some of these characters.

Percentage of Bunt

The percentage of bunt was determined by separating the total number of heads from each plant into bunt-free, partly bunted, and fully bunted groups. In computing the percentage of bunt per plant, an attempt was made to weight the results according to whether a head was fully or partly bunted. The number of fully bunted heads was multiplied by 1 and the partly bunted heads by 0.5. The sum of these products was then divided by the total number of heads, giving a percentage based on the severity of infection. The average percentage of bunt in each strain or parent-check row was then determined from the total number of plants. The percentage of bunt thus determined is considerably lower than the percentage of bunted plants. The comparable data are shown in Table 2.

TABLE 2.—*Percentage of bunt compared with percentage of infected plants in rows of the Hope and Hard Federation parents and 107 F_3 hybrid strains grown at Bozeman, Mont., in 1929*

Percentage of bunt	Number of rows of—			Percentage of bunted plants	Number of rows of—		
	Hope	F_3 strain	Hard Federation		Hope	F_3 strain	Hard Federation
2.5	5	6		2.5	3		
7.5		18		7.5	2		
12.5		17		12.5			
17.5		25		17.5			
22.5		21		22.5		1	
27.5		11		27.5		1	
32.5		6		32.5			
37.5		2	1	37.5		3	
42.5		1	1	42.5		2	
47.5			2	47.5		6	
52.5			2	52.5		5	
57.5				57.5		8	
62.5				62.5		8	
67.5				67.5		19	1
72.5				72.5		10	
77.5				77.5		25	2
82.5				82.5		9	
87.5				87.5		7	2
92.5				92.5		3	1
Total	5	107	6	Total	5	107	6
Average [a]	2.5	17.6	46.7	Average [a]	4.5	68.5	81.7
P. E.	0	±.6	±1.6	P. E.	±.8	±.9	±2.5

[a] Average percentage of bunt or bunted plants.

The data in Table 2 show that in the hybrids the average percentage of bunt was only about one-fourth as great as the percentage of infected plants. The percentage of bunt measures the effect of bunt infection on yield, whereas the percentage of infected plants is used to determine the inheritance of bunt reaction. The correlation between the percentage of bunt and the percentage of infected plants is positive, $r = +0.741 \pm 0.029$. This is both important and significant from a plant-breeding standpoint, indicating that the two measures are closely related.

Yield of Grain

The heads from each plant were threshed and the yield of grain per plant was determined in grams. The total of these yields was then divided by the number of plants in each row. There was some variation in the number of plants per row, but it was felt that the variation was not serious enough to influence the results in general. These average yields are presented in frequency classes in Table 3, where the average yields for the parent checks and hybrid strains also are shown. The average yields of the hybrids approach those of the resistant Hope parent. There was a wide and significant difference between the parents, although the average yields of the bunt-free plants of the parent checks did not show so much difference, those of Hope averaging 10.6 g[3] and those of Hard Federation 9.9 g. As shown in Table 3, the average yield of the smut-free hybrid plants was 11.8 ± 0.1 g and that of the bunted hybrid plants 8.8 ± 0.1 g. The difference of 3.0 ± 0.1 g is statistically significant and represents an average loss in yield of 20.5 per cent.

Table 3.—*Average plant yield of rows of Hope and Hard Federation parents, of 107 F_3 hybrid strains, and of infected and bunt-free hybrid plants grown at Bozeman, Mont., in 1929*

Yield (grams)	Number of rows of—				
	Hope	F_3 strains	Hard Federation	Infected hybrid plants	Bunt-free hybrid plants
4.5			3	2	
5.5		2		4	
6.5		3	3	6	
7.5		11		26	2
8.5		21		21	7
9.5	1	25		23	7
10.5	3	24		15	19
11.5	1	12		7	24
12.5		7		1	23
13.5		1		2	14
14.5		1			6
15.5					1
16.5					4
Total	5	107	6	107	107
Average [a]	10.5	9.7	5.5	8.8	11.8
P. E.	±.1	±.1	±.3	±.1	±.1

[a] Average plant yield of all rows.

[3] g is the abbreviation for gram or grams recently adopted by the Style Manual for U. S. Government printing.

The relation between the percentage of bunt and the yield per plant is shown in the correlation surface in Table 4. The correlation ($r = -0.554 \pm 0.045$) shows a negative relation between percentage of bunt and yield. This correlation coefficient also indicates that about 30.7 per cent of the squared variability of yield was caused by the variation in percentage of bunt present.

TABLE 4.—*Correlation between percentage of bunt and average yield per plant of 107 F_3 strains of Hope × Hard Federation wheat crosses grown at Bozeman Mont., in 1929*

Percentage of bunt	Number of strains having indicated average yield (grams) per plant										
	5.5	6.5	7.5	8.5	9.5	10.5	11.5	12.5	13.5	14.5	Total
2.5						1	3	2			6
7.5			1	2	3	8	1	3			18
12.5		1	1	1	6	3	4	1			17
17.5				7	11	4	2	1		1	26
22.5			5	6	2	5	2				20
27.5		1	1	2	6				1		11
32.5	1		2	3							6
37.5	2										2
42.5	1										1
Total	4	2	10	21	28	21	12	7	1	1	107

$r = 0.554 \pm 0.045$.

An effort was made to determine the decrease in yield caused by the increase of bunt. There is a strong negative relation between the percentage of bunt and yield. This correlation is not entirely correct, however, since the F_3 strains were not all genetically alike in yielding ability. Since each hybrid row contained both clean and bunted plants, it was possible to obtain a measure of the yield of each group. It was then possible to determine the percentage of loss in yield caused by the infection of bunt.

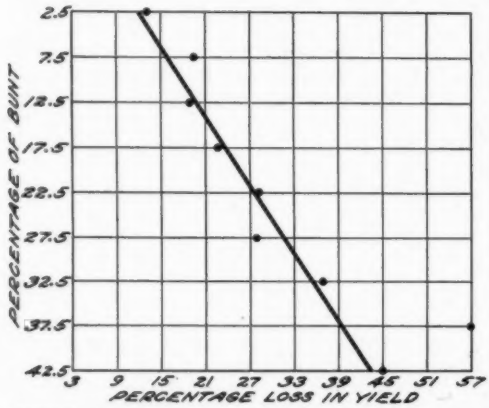

FIGURE 2.—Regression line showing relation between percentage of bunt and loss in yield. Each increase of 5 per cent in bunt caused a decrease of about 4 per cent in yield

The correlation between the percentage of bunt and the percentage loss in yield was found to be $r = +0.501 \pm 0.049$. Figure 2 shows the regression of percentage loss in yield on the percentage of bunt. Dots indicate the mean loss for each of the bunt classes. The regression line and the dots coincide very well except for the last two classes. In the 37.5 per cent bunt class there were only two observations and in the 42.5 per cent class only one. These small numbers account

in part for the variation. According to these data an increase of 5 per cent in bunt caused a decrease of about 4 per cent in yield.

Glume Color

Glume colors of wheat are most commonly classed as white and brown. Biffen (*1*) first reported brown glume color dominant to white in the single 3:1 or 1:2:1 ratio. Although there often is considerable variation in the intensity of the brown color, no other ratio has been reported for common wheats.

Glume color was not described in the F_2 generation, as the plants were grown in the greenhouse, and under such conditions the glumes of all plants were white or nearly so. In the F_3 generation the strains were recorded as brown, segregating, or white for glume color. The data are presented in Table 5.

Table 5.—*Breeding behavior in the F_3 generation for glume color of 107 strains of the Hope × Hard Federation wheat cross grown at Bozeman, Mont., in 1929*

Breeding behavior for glume color	Number of F_3 strains		Deviation
	Obtained	Calculated on 1:2:1 ratio [a]	
Brown	29	27	2
Segregating	59	53	6
White	19	27	8
Total	107	107	

[a] $P=0.21$.

These data reveal a slight shortage of white-glumed strains, although the value $P=0.21$ indicates a fairly close fit to the 1:2:1 ratio explained by a single genetic-factor difference.

Kernel Color

The inheritance of red kernel color was first reported by Biffen (*1*) as dominant over white in the F_1 generation and as segregating in a 3:1 ratio in the F_2. Nilsson-Ehle (*10*) later reported crosses which in the F_2 gave 15:1 and 63:1 ratios of red to white-kerneled plants, proving that two and three genetic factors were present. Similar ratios have since been found by other workers.

In the present study red proved dominant, as usual, in the F_1 generation, and in the F_2 generation all the plants harvested in the greenhouse had red kernels. In the F_3 the strains were described for kernel color as either red or segregating. No true-breeding white-kerneled strains were recovered. The data on segregation are presented in Table 6.

These data reveal the absence of white-kerneled strains and the presence of more true-breeding red than segregating strains. On a basis of three dominant factors for red color, 37 parts are expected to breed true for red, 8 parts to segregate 63:1, 12 parts to segregate 15:1, and 6 parts to segregate 3:1. Thus there are 26 parts segregating to 37 breeding true for red. On this calculated basis the value of $P=0.34$ indicates a fairly good fit to the 3-factor 37:26:1 ratio.

TABLE 6.—*Breeding behavior in the F_3 generation for kernel color of 107 strains of the Hope × Hard Federation wheat cross grown at Bozeman, Mont., in 1929*

Breeding behavior for kernel color	Number of F_3 strains		Deviation
	Obtained	Calculated on a 37:26:1 ratio [a]	
Red	65	62	3
Segregating	42	43	1
White	0	2	2
Total	107	107	

[a] $P=0.34$.

AWNEDNESS

Biffen (*1*) was the first to point out that the awnless condition of a wheat spike is dominant. Other early workers obtained similar results. The beardless or awnless type referred to by these investigators was evidently tip awned, corresponding to class 3, or the awnleted type, of the present study.

Howard and Howard (*9*) were the first to work with the true awnless wheats, and they reported that two factors were necessary for an interpretation of the inheritance of awns. In similar studies between Kota × Hard Federation crosses or true awnless (class 1) and fully awned (class 5) parents, Clark (*4*) concluded that two genetic factors could not entirely account for the breeding behavior.

In crosses between awnless (class 1) and awnleted (class 3) wheats, Clark and Hooker (*6*) have shown that two factors were necessary to explain satisfactorily the segregation of Hard Federation × Marquis crosses.

The inheritance of awnedness was more thoroughly reviewed and further studied by Clark, Florell, and Hooker (*5*), who classified hybrids into five classes—(1) awnless, (2) apically awnleted, (3) awnleted, (4) short awned, and (5) awned. In crosses between the awnless Bobs and Hard Federation (class 1) parents with the awned Propo (class 5) parent, imperfect dominance of awnlessness was shown as the F_1 (class 2) approached nearer the awnless than the awned parent. The F_2 and F_3 plants were separated into the five classes. The Bobs × Propo cross was less complicated in F_3 than the Hard Federation × Propo cross, the former segregating into 7 breeding groups and the latter into 11. A genetic interpretation of the results for the Bobs × Propo cross was made on the basis of two major (*AA* and *BB*) factor pairs. The corrected F_2 for the five classes was found to be close to a 1 : 8 : 4 : 2 : 1 ratio.

The study was continued in the present cross, in which Hope is awned, class 5−, and Hard Federation awnless, class 1+. The plus (+) and minus (−) signs are used to indicate the direction of overlapping. The F_2 plants of the Hope × Hard Federation cross grown in the greenhouse were carefully separated into five awnedness classes as defined by Clark, Florell, and Hooker. The data are shown in Table 7.

The F_1 and the largest number of F_2 plants were of class 2, apically awnleted. These approach more closely the awnless than the awned parent. An imperfect dominance of awnlessness is again shown,

although a larger number of awnleted (class 3) plants in the F_2 was recorded in this cross than in the other crosses studied.

TABLE 7.—*Segregation for awnedness in F_2 plants of Hope × Hard Federation crosses grown in a greenhouse at the Arlington Experiment Farm, 1928–29*

Class	F_2 plants		Class	F_2 plants	
	Number	*Per cent*		*Number*	*Per cent*
(1) Awnless	10	4.2	(4) Short-awned	18	7.6
(2) Apically awnleted	104	43.9	(5) Awned	28	11.8
(3) Awnleted	77	32.5	Total	237	100

A total of 107 F_2 plants was selected at random for growing in the F_3 generation. The material proved to be rather variable, as it was possible to separate 21 types of segregation. These are shown in Table 8. The modal classes are indicated by italic numbers. Several of these types may not differ inherently from the others. They are arranged in the order of length of awns, and totals are drawn between groups not regarded as inherently different. Variation within the groups may be due either to minor genetic factors or to environment.

TABLE 8.—*Breeding behavior of 107 F_3 strains of Hope × Hard Federation crosses at Bozeman, Mont., 1929*

Awnedness class No. of—	Number of F_3 strains grown from F_2 awnedness class No.—					Total	
	1	2	3	4	5		
1	4					4	
1, 2			1			1	
Total							5
1, 2, 3	1	2				3	
1, *2*		1				1	
1, *2*, 3	1	16				17	
2, 3		2				2	
Total							23
1, *2*, 3, 4		2				2	
1, *2*, 3, 4, 5		4	1			5	
1, 2, *3*, 4, 5		8	5			13	
2, *3*, 4, 5		1	3			4	
Total							24
2, *3*, 4		1				1	
3			7	1		8	
3, 4			3	2		5	
Total							14
2, *3*, –, 5			4			4	
3, 4, 5			5	1		6	
3, –, 5		1	6			7	
Total							17
3, *4*				1		1	
2, 3, *4*, 5				1		1	
3, *4*, 5			1	7	9	17	
3, 4, *5*					1	1	
Total							20
5					4	4	4
Total	6	38	36	13	14		107

* Modal classes are indicated by italic numbers.

There is a wide range of overlapping among the F_2 awnedness classes. Of the 6 awnless plants grown, 4 bred true and 2 segregated for classes 1, 2, and 3. Of the 14 awned plants grown, 4 bred true and 10 segregated for classes 5, 4, and 3. All the other true-breeding and segregating types are shown with the modal class indicated. There were true-breeding awnleted or class-3 strains but no true-breeding class-2 or class-4 strains.

Allowing for a wide range of overlapping of the classes, the material was separated into seven groups (Tables 8, 9, 10), i. e., (I) strains of class 1, 2, or 3 that have a mode of class-1 plants and breed true or to the limits of classes 1 and 2; (II) strains of class 2 that have a mode of class-1 or class-2 plants and segregate for classes 1, 2, and 3, or two of them; (III) strains that segregate for classes 1, 2, 3, 4, and 5, or at least four of them; (IV) strains of class 3 that breed true or have a mode of class-3 plants and do not contain plants of classes 1 and 5; (V) strains that segregate for classes 3 and 5, or have a mode of class-3 plants with only a few class-2 or class-4 plants; (VI) strains of class 4 that have a mode of class-4 or class-5 plants and segregate for classes from 2 or 3 to 5; and (VII) strains of class 5 that breed true. Table 9 shows the number of plants in each awnedness class in each of the seven groups.

TABLE 9.— *Number of F_3 plants in each awnedness class for each F_3 breeding group*

F_3 breeding groups	Number of F_3 plants in awnedness class No.—					
	1	2	3	4	5	Total
I	245	15				260
II	247	655	364			1,266
III	80	389	508	203	113	1,293
IV		15	731	47		793
V		18	639	63	233	953
VI		1	242	564	311	1,118
VII					218	218
Total	572	1,093	2,484	877	875	5,901

In Group I there were 15 plants in 260 that were classified as of class 2. In Group III, which should segregate as in the F_2 generation, there were more class-3 than class-2 plants. In Groups IV, V, and VI there was considerable variation resulting in overlapping.

In Table 10 the percentages obtained in the F_2 generation are corrected upon the breeding behavior of the F_3 strains. For example, of awnless plants (class 1) progenies of 6 of the 10 plants so classified in F_2 were grown and 4, or 66.7 per cent, bred true. This percentage of 4.2 per cent, originally classified as awnless (class 1), is 2.8 per cent. In class 3, the progeny of 1 of the 36 plants grown, or 2.8 per cent of the class, bred as Group I. This percentage of 32.5 per cent is 0.9 per cent. The total of 2.8 and 0.9 per cent gives a corrected percentage of 3.7 awnless, or Group I. Of awned plants (class 5) progenies of 14 of the 28 plants so classified were grown, and of these 4, or 28.6 per cent, bred true. This percentage of the original 11.8 per cent is 3.4 per cent. These 3.7 per cent awnless, and 3.4 per cent awned, approach 6.25 per cent, or one-sixteenth of the total. A closer approach would result by assuming from Table 8 that the variability of both these classes extends to class 3. A poorer approach would result by allowing for no variability or overlapping.

TABLE 10.—*Breeding behavior of 107 F_3 strains with the original and corrected F_2 classification of 237 plants*

F_3 breeding group	Number and percentage of F_3 strains by F_2 class No.—										Total	Corrected F_2 class	Corrected per centage F_2
	1		2		3		4		5				
	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent	Number	Per cent			
I	4	66.7			1	2.8					5	1	3.7
II	2	33.3	21	55.3							23	2	25.7
III			15	39.5	9	25.0					24	2	25.5
IV			1	2.6	10	27.8	3	23.1			14	3	11.9
V			1	2.6	15	41.6	1	7.7			17	3	15.2
VI					1	2.8	9	69.2	10	71.4	20	4	14.6
VII									4	28.6	4	5	3.4
Total	6	100.0	38	100.0	36	100.0	13	100.0	14	100.0	107		100.0
Original F_2: Total number	10		104		77		18		28		237		
Per cent		4.2		43.9		32.5		7.6		11.8			100.0

The results of these studies indicate that a genetic interpretation should be confined to two major factors. With respect to these the formula for the awnless Hard Federation parent would be *AABB*, and that for the awned Hope parent would be *aabb*. On this basis the phenotypic class, general breeding behavior, and genotypic groups may be indicated as follows:

PHENOTYPIC CLASS, BREEDING BEHAVIOR, AND GENOTYPIC GROUP

		Group
Class 1, Awnless:		
1 *AABB*	Breeding true	I
Class 2, Apically awnleted:		
2 *AABb* 2 *AaBB*	Segregating for classes 1, 2, and 3	II
$\frac{4}{8}$ *AaBb*	Segregating as in F_2 for classes 1, 2, 3, 4, and 5	III
Class 3, Awnleted:		
1 *AAbb* 1 *aaBB*	Breeding true	IV
$\frac{2}{4}$ *Aabb*	Segregating for classes 3 and 5	V
Class 4, Short-awned:		
2 *aaBb*	Segregating for classes 3, 4, and 5	VI
Class 5, Awned:		
1 *aabb*	Breeding true	VII

The goodness of fit is shown in Table 11, based on calculated numbers for two genetic factors on both 5 phenotypic classes and 7 genotypic groups. The agreement is very good but there is a slight shortage of both awnless and awned strains. The 1:8:4:2:1 phenotypic ratio is the essential explanation of inheritance in the 5 awnedness classes. The value $P=0.27$ for the genotypic ratio indicates that a worse fit, due to chance alone, might be expected 27 times in 100 trials. It would seem, therefore, that any theory assuming two major genetic-factor differences should satisfactorily explain the inheritance of awnedness in this cross. Although it is recognized that some of the variability observed may have been caused by minor factors, it has been impossible to prove the interaction of an additional genetic factor or factors because of the effects of uncontrolled variation caused by environment.

TABLE 11.—*Goodness of fit determined on a 2-factor basis for 237 F_2 plants corrected on the basis of the breeding behavior of 107 F_3 strains*

F_3 genotypic groups	Corrected F_2 class	Number of F_2 plants			
		5 phenotypic classes		7 genotypic groups	
		Obtained	Calculated [a]	Obtained	Calculated [b]
I	1	9	15	9	15
II	2	122	118	61	59
III	2			61	59
IV	3	64	59	28	29
V	3			36	30
VI	4	34	30	34	30
VII	5	8	15	8	15

[a] $\chi^2=6.76$; $P=0.15$.
[b] $\chi^2=7.57$; $P=0.27$.

No consistent or important relation was found between the seven genotypic awnedness groups and the percentage of bunt or yield per plant, as determined by correlation ratios.

SUMMARY

The parent reaction to bunt, or stinking smut, and the segregation as shown by the average of F_3 hybrid strains for three crosses, Hope × Marquis, Hope × Ceres, and Hope × Hard Federation, was studied. Hope was shown to be strongly resistant and Marquis weakly resistant, averaging 2.1 and 44.2 per cent of bunted plants, respectively. Ceres was found weakly susceptible and Hard Federation strongly susceptible with an average of 64.2 and 81.7 per cent of bunted plants, respectively. The Hope × Hard Federation cross showed a strong tendency for dominance or a preponderance of susceptibility. No F_3 strain was found within the limits of the Hope parent, indicating the presence of several genetic factors. In the Hope × Ceres cross an intermediate inheritance was indicated, with a normal curve between the parents but with considerably less than one-fourth within the limits of each parent. In the Hope × Marquis cross there was a tendency toward a dominance of the stronger resistance of the Hope parent, with only 8.7 per cent within the limits of the weak resistance of the Marquis parent. The results from the three crosses indicate that the stronger the degree of resistance involved in the hybrids the less complicated is the inheritance, with an increasing tendency away from an imperfect dominance of susceptibility toward an imperfect dominance of strong resistance.

In the Hope × Hard Federation populations the percentage of bunt was about one-fourth the percentage of infected plants. The negative correlation $r = -0.554 \pm 0.045$ was found between percentage of bunt and average yield per plant.

The correlation between percentage of bunt and percentage loss in yield was found to be $r = +0.501 \pm 0.049$. The regression of percentage loss in yield on percentage of bunt is shown. An increase of 5 per cent in bunt caused a decrease of about 4 per cent in yield.

Glume color was shown to be controlled by a single genetic-factor difference, a 1:2:1 ratio for brown, segregating, and white having been obtained.

Kernel color was found to be controlled by at least three genetic factors, with a good fit to the theoretical 37:26:1 ratio for red, segregating, and white.

The expression of awnedness was shown to be rather variable, but by grouping different segregating types a good fit was shown for five phenotypic classes and for seven genotypic groups. Two major factors are clearly shown, with a 1:8:4:2:1 phenotypic ratio for five awnedness classes. No important relation was found between awnedness on the one hand and bunt or yield on the other.

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RHABDITIS LAMBDIENSIS, A NEMATODE POSSIBLY ACTING AS A DISEASE AGENT IN MUSHROOM BEDS [1]

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INTRODUCTION

Recently, Prof. L. Haseman, of the College of Agriculture, University of Missouri, submitted larval and adult nemas that according to his observations had been destructive in mushroom beds in Leeds, Mo. Professor Haseman writes:

> Two or three weeks before the complaint was received I saw the mushroom cellar and it was in perfect condition as regards the development of the mushrooms and the complete absence of insect pests and other enemies of mushrooms. All of a sudden, apparently due to overwatering, a portion of the house showed the work of these little worms. They appeared on the beds as well as on the top of the mushroom caps. A dozen or so worms of varying sizes could be found in a little decaying pit on the top of the cap and from day to day the size of these decaying pits increased, and I take it multiplication or development of the worms continued. Eventually the entire mushroom would decay.

According to the grower, some of the beds in one cellar were practically ruined. The present paper reports the results of an investigation of this problem.

RHABDITIS LAMBDIENSIS AS A DISEASE AGENT

The "worms" submitted by Professor Haseman proved to be nemas of the species *Rhabditis lambdiensis* Maupas. Rhabditis species are usually saprophytic or saprozoic, although a few are known to parasitize man and animals and many occur in diseased plants. *R. lambdiensis* has been reported under the name *R. monhystera* Bütschli [3] (1) on decaying leaves of banana plants in Fiji, and (2) from fresh and living grass and on celery in Australia,[4] and under the name *R. lambdiensis* (3) from moist soil in three localities in French North Africa.[5] It is further known to have occurred in decaying plant material in Washington, D. C., and in Salem, Utah.[6]

POSSIBLE RELATIONSHIP BETWEEN RHABDITIS AND THE PRESENT MUSHROOM DISEASE

The association of *Rhabditis lambdiensis* with mushrooms may be interpreted in several ways. A saprophytic organism may live (1) on the contents of the cells of a dead plant (more or less decomposed),

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[1] Received for publication Aug. 10, 1932; issued April, 1933.

[2] For the literature references citing the works of Falconer and of Elliott (footnotes 7 and 8) the writer is indebted to Neil E. Stevens and E. B. Lambert, of the Division of Mycology and Disease Survey, Bureau of Plant Industry, U. S. Department of Agriculture.

[3] COBB, N. A. NEMATODES, MOSTLY AUSTRALIAN AND FIJIAN. *In* Fletcher, J. J. The Macleay Memorial Volume, p. 252–308. Sydney. Reprinted in N. S. Wales Dept. Agr., Misc. Pub. 13: 29. 1893.

[4] COBB, N. A. Op. cit.

[5] MAUPAS, E. ESSAIS D'HYBRIDATION CHEZ DES NEMATODES. Bul. Biol. France et Belg. 52: 466–498, illus. 1919. In connection with the taxonomic position of the present species, compare also:
MAN, J. G. DE. DAS MÄNNCHEN DER RHABDITIS MONHYSTERA BÜTSCHLI. Zool. Anz. 70: 51–57, illus. 1926.

[6] Oral communications by B. G. Chitwood, of the Zoological Division, Bureau of Animal Industry, and G. Thorne, of the Division of Nematology, Bureau of Plant Industry, U. S. Department of Agriculture.

(2) on the substance of the cell walls, (3) on the bacteria or fungi associated with decay, or (4) on waste material due to decay-producing organisms.

In view of the fact that some Rhabditis species are known to feed exclusively on fungi, bacteria, or gelatinous products of bacteria, it seemed possible that this might be true of *Rhabditis lambdiensis* also. To determine this, the contents of the intestinal tract of a number of specimens were carefully examined. Agglomerations or balls of bacteria were found in the pharynx and in the anterior portions of the intestine. This led to the conclusion that *R. lambdiensis*, if not the primary cause of the present mushroom disease, may act as a carrier of pathogenic bacteria. In 1910 William Falconer [7] mentioned a mushroom disease identical with the one here dealt with, or at least very similar to it. He calls it "black spot," and considers the cause to be eelworms (Anguillulae). He explains that brown spots, streaks, or freckles appear on the top of the mushroom caps, increasing in distinctness and breadth with age. The presence of nemas in all these spots led Falconer and those whom he consulted to believe that these Anguillulae were the cause of the disease. The blackspot or brownspot disease of mushrooms was later recognized as of bacterial origin by various authorities.[8] It therefore seems that the pathogenic agent, *Bacterium tolaasi* (Paine),[9] may act alone or together with nemas. It is impossible from Falconer's account to recognize the nemic species involved. The term "Anguillulae" applied by him undoubtedly means nemas, or eelworms, in general, and is not a plural of the generic name Anguillula, which includes only the vinegar eel and a few related forms. It is therefore possible that Falconer's eelworms were similar to *R. lambdiensis* or identical with it.

The statement of Falconer and his associates, as well as Professor Haseman's observations on the relationship of the nemas to the disease, are undoubtedly pertinent. The nema, however, is the secondary agent. Nevertheless, in the present case it almost equals in importance the primary cause of the disease. It has been repeatedly pointed out that saprophytic and saprozoic nemas are important agents in the distribution of bacterial and fungous diseases of plants. It is known that these nemas travel readily on moist surfaces. Since mushroom beds must be sprinkled regularly, there is an opportunity for the Rhabditis to spread quickly, carrying along the pathogenic bacteria attached to the body surface or the oral opening, in the pharynx (the contents of which are sometimes emitted again), or in the intestine, from which they are discharged with the feces.

MORPHOLOGY OF RHABDITIS LAMBDIENSIS

It will be seen that the present description differs somewhat from that of Maupas [10] and also from that of Cobb [11] for *Rhabditis monhystera* (declared by Maupas to be a synonym of *R. lambdiensis*) from Fiji and Australia, especially in regard to the structure of the lip region and the arrangement of the bursal ribs. It is, however, thought that these slight differences may represent individual variations or difficulties of observation, especially of the lip region.

[7] FALCONER, W. MUSHROOMS: HOW TO GROW THEM: A PRACTICAL TREATISE ON MUSHROOM CULTURE FOR PROFIT AND PLEASURE. p. 124-126. New York. 1910.
[8] ELLIOTT, C. MANUAL OF BACTERIAL PLANT PATHOGENS. p. 227. Baltimore. 1930.
[9] ELLIOTT, C. Op. cit., p. 226-227.
[10] MAUPAS, E. Op. cit.
[11] COBB, N. A. Op. cit.

THE CUTICLE

The cuticle is rather complicated because the annules are reticulated (areolated) and have points, granules, and ridges that show considerable variability, especially in different regions of the body. Furthermore, these cuticular structures seem to vary in individuals. The regular reticulation, for example, extends much farther back in some specimens than in others. Immediately posterior to the lip region, the cuticle is simply annulated. The reticulation begins in the latitude of the anterior end of the esophagus. Here one sees the beginning of an areolation due to the appearance of small "shields" with rounded posterior edges, seen with remarkable plainness when the animal moves. Soon quadrangular areolae appear (figs. 1, F, and 2, A–H) accompanied by one or two points or rods; still farther back the areolation is not apparent but may reappear toward the tail end. However, transverse series of points may be seen all along the body, sometimes distributed rather profusely and irregularly. (Fig. 2, A–H.) On the lateral surface of the anterior portion of the body, at least, longitudinal crenate wings can be seen in considerable numbers. From optical profile views it is concluded that the points mentioned above are not surface structures but are located under the first cuticular layer. (Fig. 2, B, D, F, and H.)

DEIRIDS AND PHASMIDS

A deirid or cervical papilla was seen in the latitude of the nerve ring. As it did not protrude, it was recognized only when seen in profile. (Fig. 2, K.) On the male occurs a similar papilla, about three spicula lengths in front of the anus. (Fig. 2, L.) This was at first thought to be one of a series of lateral papillae, such as are found in other nemic forms, but no such series was located. Phasmids were not seen, although they were probably present. Maupas's drawing could be interpreted as having them.

THE LIP REGION AND ITS SENSE ORGANS

All the writer's specimens had 6 lips, each with somewhat angular outlines. (Fig. 1, A, B, and F.) Undoubtedly Maupas was mistaken in assuming the presence of only 3 lips. His drawing of the frontal view of the head shows 3 groups of 2 lips. In the present case and as here figured, no grouping was seen. Contrary to Maupas's [12] account and contrary also to Cobb's [13] descriptions of his *Rhabditis monhystera*, in which mention is made of only 1 setalike papilla for each lip, 3 papillae were seen on each submedial lip and 2 on each lateral lip. In front view, each submedial lip showed a group of 2 papillae belonging to an outer circlet and 1 papilla belonging to an inner circlet. The lateral lips, however, have apparently only 2 papillae, an inner and an outer one, both rather indistinct. The papillae are not setiform, but simply minute elevations. Inasmuch as Maupas's drawings show setiform papillae there is a possibility that the present form is different.

The lips are separated by rather wide and apparently deep furrows which lead outward to rounded excisions (fig. 1, B), intensifying the angular appearance of the lips. The amphids, not hitherto described for the present species, seem papillate if superficially seen. Close

[12] MAUPAS, E. Op. cit.

[13] COBB, N. A. Op. cit.

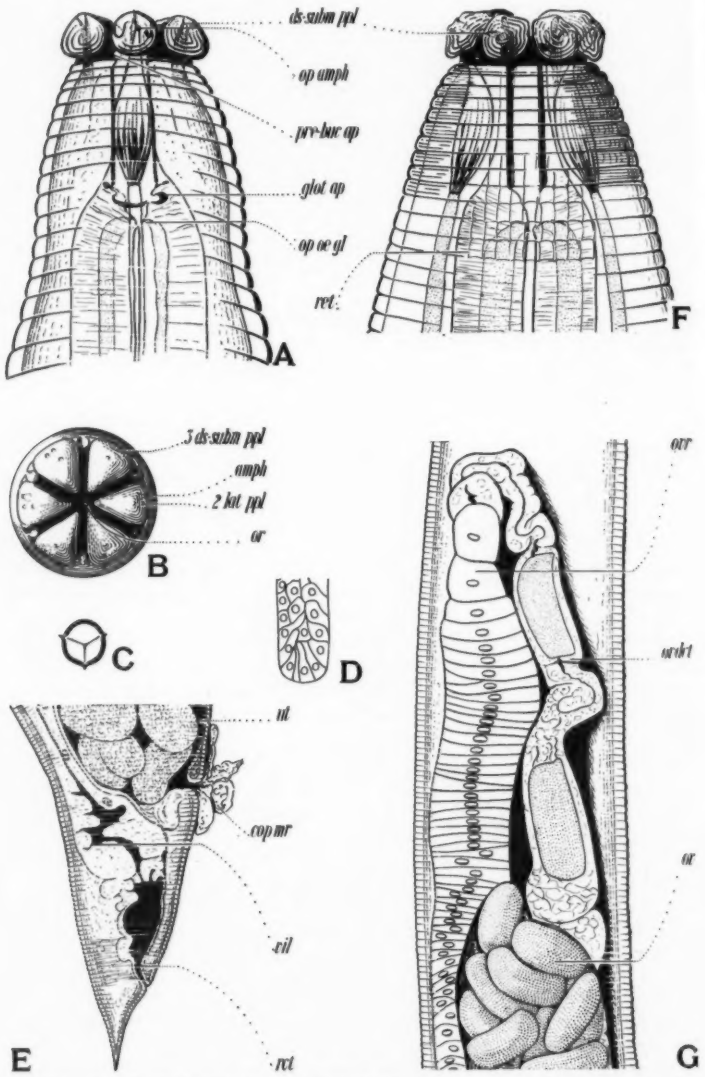

FIGURE 1.—*Rhabditis lambdiensis*, female: A, Lateral view of head end: *ds-subm ppl*, dorsosubmedial papilla; *glot ap*, glottoid apparatus; *op amph*, opening of the amphid; *op oe gl*, opening of an esophageal gland; *pre-buc ap*, prebuccal apparatus. × about 1,600. B, Front view of head: *amph*, amphidial opening; 3 *ds-subm ppl*, 3 dorsosubmedial papillae; *2 lat ppl*, 2 lateral papillae, *or* mouth opening. × about 1,600. C, Optical cross section through buccal cavity. Free-hand sketch. D, Blind end of ovary. Free-hand sketch. E, Posterior end of body: *cop mr*, copulation marks; *rct*, rectum; *ut*, uterus; *vil*, villilike process. × about 250. F, Dorsoventral view of head end: *ds-subm ppl*, dorsosubmedial papilla; *ret*, reticulation of the cuticle. × about 1,600. G, Region of the anterior oviduct: *ov*, egg in uterus; *ovdct*, oviduct; *ovr*, ovary. × about 250

observation shows that they open slightly dorsad from the outer lateral papillae. Figure 1, A and F, shows side and profile views of them. The so-called amphidial pouch (sensilla) is apparently gourd-shaped or flask-shaped, extending inward to nearly the beginning of the esophageal region and widening in the postlabial portion. A number of nerve terminals were faintly discernible inside the pouch. Posteriad this structure connects with its nerve, which by close observation could be followed nearly to the nerve ring.

THE ALIMENTARY TRACT

The vestibule consists of a cuticular apparatus at the base of the lip region, which can be seen best on living specimens, preferably in side

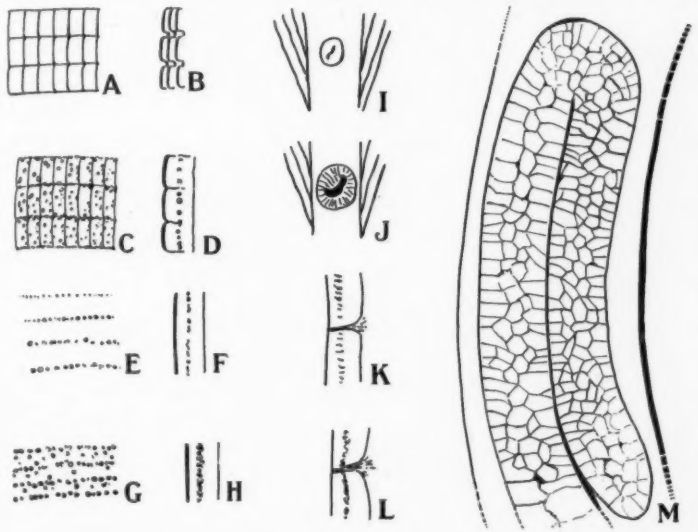

FIGURE 2.—*Rhabditis lambdiensis*: A, Surface view of reticulation of cuticle, anterior to median bulb; B, profile view of A; C, surface view of reticulation of cuticle, near excretory pore; D, profile view of C; E, surface view of reticulation of cuticle, middle region of the body; F, profile view of E; G, surface view of reticulation of cuticle, in front of spicula; H, profile view of G; I, surface view of excretory pore; J, deeper focus on excretory pore; K, deirid, profile view; L, lateral papilla on male, three spicula lengths in front of anus; M, blind end of testis. A–L, Free-hand sketches; M, × about 370

view; its parts closely resemble teeth, especially when moving. However, its function may be mainly that of supporting the exceedingly mobile lip region. Doubtless in structure and function this prebuccal apparatus is similar to, if not homologous with, the so-called glottoid apparatus behind the buccal cavity. Both are very mobile sections separating more rigid parts. The walls of the buccal cavity are not so thick as in many other species of Rhabditis. There is an encircling ring at the beginning of the last fourth of the cavity and another at the end of the first fourth. These rings probably are markings, the former produced by muscle attachments and the latter by the attachment of the esophagus.

The esophagus looks like that of other Rhabditis species. There are 3 esophageal or salivary glands, 1 dorsal and 2 ventrosubmedial.

The nucleus of each is located in the cardiac bulb. A narrow canal with slightly thickened walls leads from each gland anteriad through the isthmus; as the canals reach the oval medial bulb the thick lining disappears, and they seem to widen to a cylindrical granulated portion traversing this bulb and connecting anteriad with a canal of thickened walls which empties into the esophageal lumen a short distance behind the glottoid apparatus. It appeared at first as if the 3 glands emptied into the esophagus where the cuticularized canals left the isthmus and entered the medial bulb; but a more detailed study proved that the granular portions in the medial bulb were apparently connected with the canals.

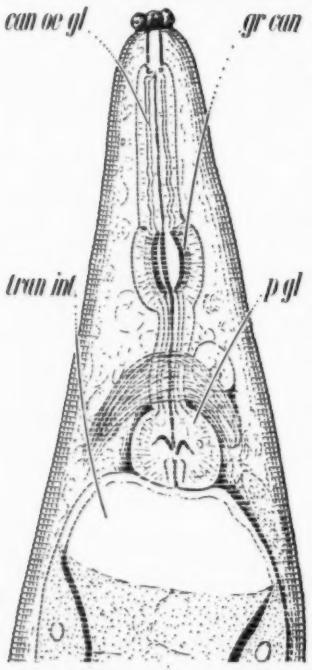

FIGURE 3.—*Rhabditis lambdiensis*, anterior portion of a female: *can oe gl*, canal leading from esophageal or salivary glands; *gr can*, cylindrical granulated portion of canal; *p gl*, position of the nuclei of esophageal or salivary glands; *tran int*, transparent portion of intestinal cells. × about 370

Six cells form the circumference of the intestine. The initial portion of the intestine (fig. 3) is lighter colored and much more transparent than the rest, as the cells in that region are thinner than those posteriad. The intestinal lumen in the prerectal portion is highly sinuate, the individual cells often forming villilike processes extending into the intestinal cavity. A "Stäbchensaum" is well developed along the entire intestine. Proximad the rectum has the usual three rectal cells.

THE EXCRETORY SYSTEM

Maupas described the excretory system of the present form as being of the H type, consisting of two anterior and two posterior lateral branches. The observations of the writer seem to confirm this; the canals, however, were seen only a short distance and are sinuate. A ventral view of the common outlet showed what seem to be radial muscular fibers around its proximal portion (fig. 2, J), where it is somewhat wider than at its intersection with the cuticle. It is possible that this may be the portion that serves as the "pulsating" vesicle seen in certain other species of Rhabditis.

THE FEMALE SEXUAL APPARATUS

The female sexual apparatus is of typical prodelphic character. There is practically no sign of a posterior uterine branch; the anterior one, however, is very large and in adult females is filled with numerous eggs which sometimes contain fairly advanced embryos. There is a distinct oviduct (fig. 1, G); its end portion is reflexed and connects with the ovary, which in some instances was seen to extend back

almost to the vulva. The end of the ovary is shown in Figure 1, D; no differentiated terminal cell was seen. Some of the females exhibited so-called copulation marks, i. e., the vulvar opening was covered with a hardened brownish mass, the remains of a cement apparently produced by the male during copulation.

THE MALE SEXUAL APPARATUS

The male genital system has a reflexed testis similar in structure to the ovary. The copulatory apparatus is highly complex because of a

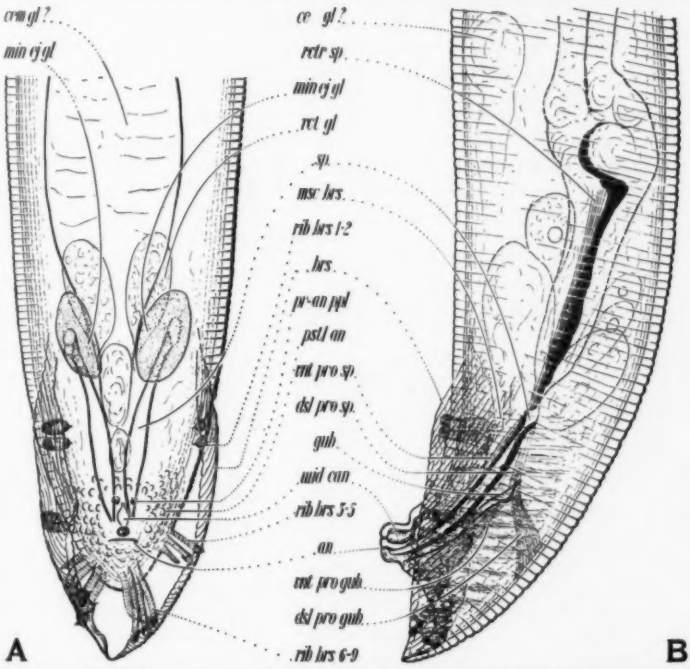

FIGURE 4.—*Rhabditis lambdiensis*, posterior portion of male: A, Ventral view; B, lateral view. *an*, anus; *brs*, bursa; *cem gl* ?, cement gland (?); *dsl pro gub*, dorsal protrudor muscle of gubernaculum; *dsl pro sp*, dorsal protrudor muscle of spicula; *gub*, gubernaculum; *min ej gl*, small ejaculatory gland (2); *msc brs*, muscles of bursa; *pr-an ppl*, preanal papilla (2); *pstl an*, pustules surrounding anal region; *rct gl*, rectal gland (3); *retr sp*, retractor muscles of spicula; *rib brs 1–2*, ribs of bursa 1–2; *rib brs 3–5*, ribs of bursa 3–5; *rib brs 6–9*, ribs of bursa 6–9; *sp*, spicula; *vnt pro gub*, ventral protrudor muscle of gubernaculum; *vnt pro sp*, ventral protrudor muscle of spicula; *wid can*, widening of outlet canal. × about 720

number of accessory organs. There is a pair of almost straight spicula (fig. 4, A and B) slightly cephalated at the inner end, the whole somewhat resembling the blade of a knife. A single gubernaculum was seen, similar in shape to the spicula, but only two-thirds as long. (Fig. 4, B.) The following muscles were connected with this spicular apparatus: (1) A long retractor muscle attached to the inner end and running obliquely forward to the dorsolateral body wall, (2) a ventral protrudor muscle attached also to the inner end of the spicula and to the ventral body wall in front of the anus, (3) a dorsal protrudor muscle connecting the inner spicula ends with the proximal end of the gubernaculum,

(4) a ventral protrudor gubernaculi connecting the inner end of the gubernaculum and the ventral body wall back of the anus, and (5) a dorsal protrudor gubernaculi attached to the inner end of the gubernaculum and to the dorsal body wall.

As shown in Figure 4, B, there is also a series of so-called bursal muscles present, extending from near the anterior end of the bursa to the middle of the tail. The bursa has very typical characters. It begins slightly in front of the spicula and surrounds the tail completely. (Fig. 4, A and B.) Its surface structure is much like the cuticle, although not so regular. Figure 4, A and B, shows the striation of such a bursal wing. The ribs as seen in all the present material have a slightly different arrangement from that figured by Maupas [14] and that sketched by Cobb [15] for his Australian specimens. There were probably 9 ribs in each of the specimens. Cobb mentions only 8 for his specimens, although his drawings show that there might have been 9. However, one of the group of 5 close to the tail end is very difficult to see and perhaps escaped the observer's notice. There is a pair of ribs in the latitude of the middle of the spicula. This pair seems to be constant and has been reported at the same place by all observers. Both ribs reach the edge of the bursa and each has a distinct terminal papilla. The second group of ribs is about in the latitude of the anus or slightly in front of it. According to Maupas's description, there are also 2 ribs in this group; this agrees with observations of the writer. In one of the specimens (fig. 4, A), however, 3 ribs were seen in this group in the left bursal wing. This is very significant because the forms described by Cobb from Australia seemed to have this as the normal number. It is therefore concluded that this species varies in this character. The last group, if normal, consists of 5 ribs which are close to the tail end. Maupas draws these ribs as slightly spaced, but in the writer's material they were close together. (Fig. 4, A.) Possibly there is some variation also in this regard. One rib, which seems to fluctuate somewhat between the second and the third group, apparently is only half as long as the others and ends on the outer surface of the bursa. Moreover, this rib differs from the others in tapering distally. The anterior rib of the second group likewise ends on the outer surface of the bursa, with a distinct point; the posterior one, however, extends to the rim of the bursal wing. The 4 posterior ribs of the third group likewise differ in this respect; the first one extends to the bursal rim, the second does not quite reach it and is seemingly turned outward, the third reaches the bursal rim, and the fourth ends on the outer surface apparently about halfway to the rim. Each of these 4 ribs ends with what seems to be a pointed papilla.

The anal region of the male, especially the part in front of the anus, protrudes in a most remarkable way (fig. 4, B); a broad elevation is formed through which penetrates the outlet of what seems to be the cement gland. In ventral as well as profile view one observes an ampullalike widening of the outleading canal. (Fig. 4, A and B.) It is, however, difficult to connect this with the cement gland itself, which seems to be a large body located ventrad of the intestine and ejaculatory duct in front of the spicula. It is rather strange that this gland should be single in the present species, whereas it is paired in

[14] MAUPAS, E. Op. cit.

[15] COBB, N. A. Op. cit.

other species of Rhabditis. Chitwood [16] points out that in some species of Rhabditis there exist still other glands in this region which in structure can not be differentiated from the cement glands and that it is not even established which of the glands produce the cement. He therefore designates the large pair (the "cement glands" of other authors) as "large ejaculatory glands" and the others as "small ejaculatory glands."

In the present species, two small ejaculatory glands are present (fig. 4, A) in addition to the triple rectal glands.

At present *Rhabditis lambdiensis* is the only species that has a preanal outlet for the cement gland opening on top of a large papillate elevation.

Still another rather remarkable character typical of this species is the presence of a considerable number of small round pustules arranged in circular series surrounding the anal opening.

In front of the papillate elevation previously mentioned is what seems to be a pair of preanal papillae, structures not hitherto seen in Rhabditis. They are rather obscure and are seen distinctly only in a ventral view. These papillae may be the openings of the two small ejaculatory glands.

SUMMARY

Rhabditis lambdiensis Maupas is described as a probable carrier and distributor of a pathogenic agent (*Bacterium tolaasi*) in mushroom beds and cellars. Its relationship to the so-called black-spot or brown-spot disease of cultivated mushrooms is discussed. A detailed description of the nema is given, including new data on the morphology of this species and on that of the genus in general.

[16] CHITWOOD, B. G. STUDIES ON SOME PHYSIOLOGICAL FUNCTIONS AND MORPHOLOGICAL CHARACTERS OF RHABDITIS (RHABDITIDAE, NEMATODES). Jour. Morph. and Physiol. 49: 251–275, illus. 1930.

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ELIMINATION OF NATURAL MORTALITY AS A FACTOR IN DETERMINING THE EFFECTIVENESS OF HYDROCYANIC ACID GAS ON THE CALIFORNIA RED SCALE[1]

By F. S. STICKNEY, *Associate Entomologist*, and H. R. YUST, *Senior Scientific Aid, Division of Fruit and Shade Tree Insects, Bureau of Entomology, United States Department of Agriculture*

INTRODUCTION

In tests on the fumigation of the California red scale (*Chrysomphalus aurantii* Mask.) with hydrocyanic acid gas, natural mortality as ordinarily considered has ranged from 1 to 71 per cent. This has introduced so large an error in experimental tests as to render precise results almost impossible. Therefore, an attempt has been made to establish qualitative tests, based on such phenomena as changes in color and conformation, by which the scales killed by treatment could be distinguished from those that died naturally. Though a complete separation has not been possible, the number of dead scales occurring on untreated, or check, lemons which are indistinguishable from those killed on recently fumigated fruit have proved to be few. However, it has been found that the scales dying from natural causes bear a nearly constant ratio to the number of living scales plus the number of dying scales. This paper is concerned with the amount of fluctuation that one may expect in natural mortality, as defined by these new criteria.

When the California red scale is killed by treatment with hydrocyanic acid gas, it becomes darker in color and then gradually dries out. Scales dying naturally go through a similar change. An attempt was made to determine what factors control the numbers of these dying scales. To simplify the problem, it was decided to use only lemon fruits, and to make counts exclusively of the female in the later periods of her development. At this time the female is relatively large and bodily changes can be noted with less chance of error than is possible during the earlier periods of her development. She is also more resistant to hydrocyanic acid gas. The stages used were the fully developed second stage, the gray adult, and the mature adult.[2] On dying, the scales in these three stages first grow darker. Color changes are practically completed before the drying-out process begins. The color of the insect during the fully developed second stage becomes a dull brown to a dark reddish brown, that of the gray adult a dull gray to almost black, that of the mature adult a dirty reddish to blackish red brown. In drying out, the individuals become increasingly viscous, as can be recognized by probing but by no other means, so far as the writers know, up to the time they are nearly dry. Shriveling may or may not accompany the drying-out process.

[1] Received for publication June 3, 1932; issued April, 1933.

[2] The term "fully developed second stage" is applied to that part of the second stage from the time the individual has reached approximately its largest diameter and is becoming shiny, but before the derm has thickened, up to ecdysis; "gray adult" is applied to that part of the third or adult stage from the time of ecdysis until the individual has reached approximately its largest diameter and is becoming shiny and beginning to darken; "mature adult" is applied to the adult stage beyond the gray adult one.

The experimental data show that the ratio of dying to living plus dying scales [3] of the total number in the three periods from untreated fruits in fair condition varies but little, even though the factors involved may differ. This is not true for the ratio of living or of dead scales to the total scale count, where those already dead and dried out are also included. The ratio of dying scales to living plus dying scales on untreated fruits shows so little variation that by taking the weighted mean of a moderate number of samples a satisfactory value for expressing mortality can be obtained. This value may then be used in eliminating the natural mortality from counts on treated samples. The terms "mortality" and "natural mortality" occurring hereafter, when used without explanation, refer to the ratio of dying scales to living plus dying scales present, expressed as a percentage.

STABILITY OF MORTALITY VALUES ON UNTREATED CHECKS

To be of broad applicability for experimental tests, the value for expressing natural mortality should be satisfactorily stable under a variety of conditions encountered in practice. The writers have found that the following factors do not definitely influence this value as obtained by their method: Different locations on the same tree; corresponding locations on different trees; different locations on different trees; size of fruit (samples [4] ranged from 1⅛ to more than 2 inches long); severity of infestation (single samples ranged in total counts from 16 to 600 scales); protection by overhanging foliage (records were from a grove rather sparsely foliated); time of year (December 3, 1930, to January 12, 1931, for one grove, and January 22 to May 21, 1931, for another); and time and conditions of storage after collection (from none to 50 days under temperature conditions, all approximately uniform, ranging from 13° to 26° C.). As for fruits of varying conditions, counts from those scales that are beginning to dry out noticeably still show the same general mortality at first, but eventually the mortality rises rapidly. In comparing mature or yellow fruits of good condition with immature or green fruits of like condition, the writers have observed a slightly higher mortality for the yellow fruits in the average of a considerable number of counts. The above-mentioned conclusions have been drawn from a total count of almost 25,000 scales, where all factors except the one under investigation were made as nearly alike as possible.

To give an idea of the stability of the mortality values obtained by the writer's method as compared with the usual practice of obtaining the net mortality of treated material from the ratio of live scales to the total number of scales on checks, two frequency distributions have been plotted. (Fig. 1.) The data were obtained from counts on 63 lemons, all green or immature, in good condition and collected from a single grove during two periods in 1931, that is, from January 22 to February 20 in the winter and from May 8 to 21 in the spring. The collections in the winter period were stored from 3 to 21 days at 13° C., those in the spring period were not stored. Counts of the latter were made on the day the samples were collected or on the following day. The abscissa of the chart gives, in terms of percentage, the ratio of living to living plus dying

[3] All scales discolored or in process of drying out are referred to as dying. Living scales denote live scales exclusive of those dying.

[4] In this paper a sample refers to an individual lemon fruit.

scales obtained from the formula $\frac{A}{A+Dy}$, and the ratio of living to total number of scales (excluding empty shells) obtained from the formula $\frac{A}{A+Dy+Dr}$, in which A refers to living scales, Dy to dying scales, and Dr to dead, dried scales. The percentage of mortality, expressed by the formula $\frac{Dy}{A+Dy}$, is, of course, obtained by subtracting the value for $\frac{A}{A+Dy}$ from 100 per cent. The abscissa is

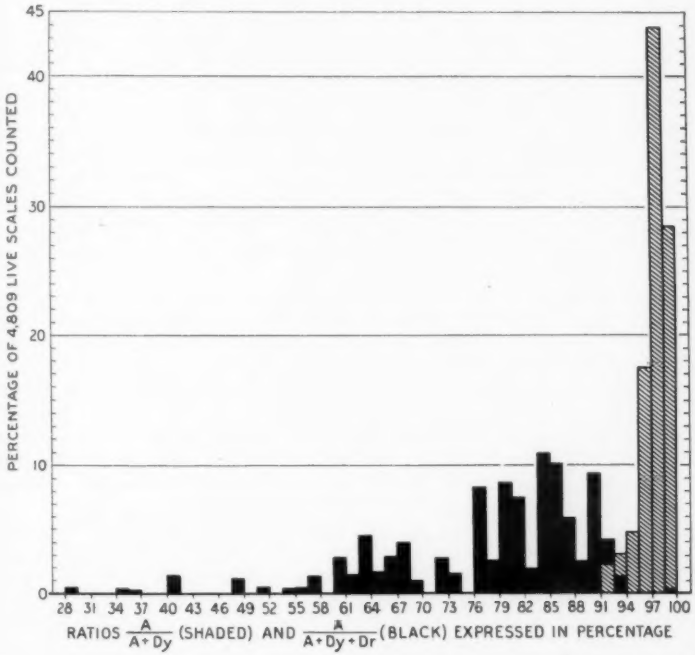
PERCENTAGE OF 4,809 LIVE SCALES COUNTED
0
10
20
30
40
45
28 31 34 37 40 43 46 49 52 55 58 61 64 67 70 73 76 79 82 85 88 91 94 97 100
RATIOS A/A+Dy (SHADED) AND A/A+Dy+Dr (BLACK) EXPRESSED IN PERCENTAGE

FIGURE 1.—Comparison of variability of the ratio of living scales to living plus dying scales $\left(\frac{A}{A+Dy}\right)$ and the ratio of living scales to the total number of scales $\left(\frac{A}{A+Dy+Dr}\right)$ from the same counts

divided into 1.5 per cent intervals. The numbers of live scales on each sample which fell in each 1.5 per cent interval on the abscissa were totaled and the ratio of this total to the total count of 4,809 live individuals, expressed as a percentage, was placed in the appropriate interval on the abscissa and read from the scale on the ordinate. This was done both for the ratio of living to living plus dying scales (shaded columns) and for the ratio of living to living plus dying plus dead and dried scales (black columns). It is seen that the range of mortality obtained by the writers' method is narrow (0 to 9 per cent), whereas that obtained by the usual method is so wide as to introduce a real error in its use.

TABLE 1.—*Comparison of variability of the ratio of living scales to living plus dying scales* $\left(\frac{A}{A+Dy}\right)$ *and the ratio of living scales to the total number of scales* $\left(\frac{A}{A+Dy+Dr}\right)$ *from counts on fruits with the following factors involved: Grove from which collected, period of collection, storage temperature, and condition of fruit*

Grove	Period of collection	Group No.	Fruits used	Storage temperature	Condition of fruit	Scales				Ratios		
						A	*Dy*	*Dr*	*A+Dy+Dr*	$\frac{A}{A+Dy}$	$\frac{Dy}{A+Dy}$	$\frac{A}{A+Dy+Dr}$
			Number	*° C.*		*Number*	*Number*	*Number*	*Number*	*Per cent*	*Per cent*	*Per cent*
	Jan. 22 to Feb. 20, 1931	1	20	13	Good (green)	2,793	69	576	3,438	97.6	2.4	81.2
	May 8–21, 1931	2	43	(a)	do	2,016	51	882	2,949	97.5	2.5	68.4
	Jan. 22 to Feb. 20, 1931	3	5	13	Good (yellow)	1,284	64	360	1,708	95.3	4.7	75.2
A	Jan. 22, 1931	4	4	13	Drying	931	28	224	1,183	97.1	2.9	78.7
	May 21, 1931	5	6	(a)	do	541	17	591	1,149	97.0	3.0	47.1
	Jan. 22 to Feb. 20, 1931	6	3	26	Good (green)	680	14	117	811	98.0	2.0	83.8
	Mar. 30, 1931	7	33	13, 17, 20, 23, 26.	Good (green or yellow or partly both)	2,092	95	1,776	3,963	95.7	4.3	52.8
		8	47	10	Good (green)	3,898	217	1,807	5,922	94.7	5.3	65.8
B	Dec. 3, 1930, to Jan. 12, 1931	9	13	10	Good (yellow)	864	49	912	1,825	94.6	5.4	47.3
		10	10	26	Good (green)	1,098	50	488	1,636	95.6	4.4	67.1
C	Jan. 26, 1931	11	3	13	Good (yellow)	184	8	21	213	95.8	4.2	86.4

[a] Counted promptly after collection.

In Table 1 a series of total counts is presented to show the relation of the mortality ratios to date of collection, temperature at which the fruit was stored (if the counts were not made promptly after collection), and condition of fruit, in three widely separated groves in the Whittier (Calif.) district. Considering the data from grove A, it is seen that counts of fruits collected between January 22 and February 20 and stored from 2 to 10 days in a relatively cold medium (Group 1) show practically the same mortality as counts from fruits collected 3 months later and not stored (Group 2); that counts on fruits stored for about the same length of time in a distinctly warmer environment (Group 6) show approximately the same mortality as those stored at the lower temperature (Group 1). This is true also for fruits distinctly drying (Group 5) when compared with fruits in good condition (Group 2). The mature fruits (Group 3) are seen to have a slightly higher scale mortality than comparable green fruits in good condition (Group 1). Group 7 represents the data for those fruits collected at the time indicated which did not dry out. The mortality on these fruits, which were stored from 8 to 50 days, was but little higher than that on the fruits in any other group and was slightly less than that on the mature or yellow fruits. The range in mortality for the seven groups in grove A is only 2.7 per cent, whereas the ratios of live scales to total scale count show a range more than twelve times as great, or 36.7 per cent.

The mortality data for groves B and C are in harmony with the corresponding data for grove A. Grove B had been much neglected. The fruits were collected earlier than those from grove A and the storage conditions were not under such satisfactory control as for the fruits from groves A and C; also the humidity was lower in both storage media. Any or all of these factors may have exerted an influence in producing a slightly higher mortality on fruits from grove B. When the counts on fruits composed of groups most nearly equivalent from groves A and B were compared, however, the difference in mortality was found to be only 2.3 per cent (Table 2), but the percentage of live scales in the total scale count differed by 13.5.

TABLE 2.—*Comparison of the ratio of living scales to living plus dying scales* $\left(\frac{A}{A+Dy}\right)$ *and the ratio of living scales to the total number of scales* $\left(\frac{A}{A+Dy+Dr}\right)$ *from combined counts on fruits consisting of groups most nearly equivalent from two different groves*

Grove	Period of collection	Group No.	Fruits used	Scales				Ratios		
				A	*Dy*	*Dr*	*A+Dy+Dr*	$\frac{A}{A+Dy}$	$\frac{Dy}{A+Dy}$	$\frac{A}{A+Dy+Dr}$
			Number	*Number*	*Number*	*Number*	*Number*	*Per cent*	*Per cent*	*Per cent*
A	Jan. 22 to May 21, 1931.	1, 2, 3, 6	71	6,773	198	1,935	8,906	97.2	2.8	76.0
B	Dec. 3, 1930, to Jan. 12, 1931.	8, 9, 10	70	5,860	316	3,207	9,383	94.9	5.1	62.5
	Total or weighted mean			12,633	514	5,142	18,289	96.1	3.9	69.1

The mortality in the three periods, when considered separately, was found to check well with the combined mortality. The data in

Table 3 were obtained on fruits in like condition collected from one grove on two different occasions. In both cases the mortality was slightly lower for the fully developed second stage than for the two adult stages. The results for both gray adult and mature adult were somewhat different for the two sets of observations, but the average mortalities derived from the sum of the two sets of figures were practically the same for both stages.

TABLE 3.—*Comparison of variability of the ratio of living scales to living plus dying scales* $\left(\frac{A}{A+Dy}\right)$ *and the ratio of living scales to the total number of scales* $\left(\frac{A}{A+Dy+Dr}\right)$ *from counts of female scales in the three later periods on fruits comparable in condition but collected at two different occasions from the same grove*

Period of collection	Scales							
	Fully developed second stage				Gray adult			
	A	*Dy*	*Dr*	*A+Dy+Dr*	*A*	*Dy*	*Dr*	*A+Dy+Dr*
1931	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Jan. 22	711	11	64	786	958	36	310	1,304
May 19 to 21	844	16	290	1,150	1,079	25	718	1,822
Total or weighted mean	1,555	27	354	1,936	2,037	61	1,028	3,126

Period of collection	Scales—Continued				Ratios					
	Mature adult				$\frac{A}{A+Dy}$			$\frac{A}{A+Dy+Dr}$		
	A	*Dy*	*Dr*	*A+Dy+Dr*	Fully developed second stage	Gray adult	Mature adult	Fully developed second stage	Gray adult	Mature adult
1931	*Number*	*Number*	*Number*	*Number*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Jan. 22	964	23	183	1,170	98.5	96.4	97.7	90.5	73.5	82.4
May 19 to 21	401	19	303	723	98.1	97.7	95.5	73.4	59.2	55.5
Total or weighted mean	1,365	42	486	1,893	98.3	97.1	97.0	80.3	65.2	72.1

In Figure 2 six frequency distributions have been plotted, in the same manner as in Figure 1, to illustrate variability of $\frac{A}{A+Dy}$ as related to the grove from which the fruit was collected, period of collection, whether or not the fruit was stored, and, if so, at what temperature. The first four distributions (A to D, inclusive) are represented by Groups 1, 2, 7, and 8, respectively, in Table 1, and the fifth and sixth distributions (E and F) by the data for groves A and B, respectively, in Table 2. The percentages for the other groups in Table 1 are not plotted, since the number of samples was too small to bring out any significant results. However, Groups 3 and 6, and 9 and 10, are included in frequency distributions E and F, respectively.

The general trends indicated in the six frequency distributions may be stated briefly as follows: In the aggregate practically the same percentage of live scales occurs in A and B, but in A the percentage falling in the interval 97 to 98.5 per cent is more than three times that in any other interval, those in the intervals on either side of this one are nearly equal, and there is a sharply reduced percentage in the next lower interval; whereas in B the largest percentage, which is not twice that of any other interval, falls in the interval 98.5 to 100 per cent, and there is a decreasing percentage in the lower intervals. In C no interval shows a decided concentration of scales, the percentages being fairly evenly distributed in the six intervals extending from 91

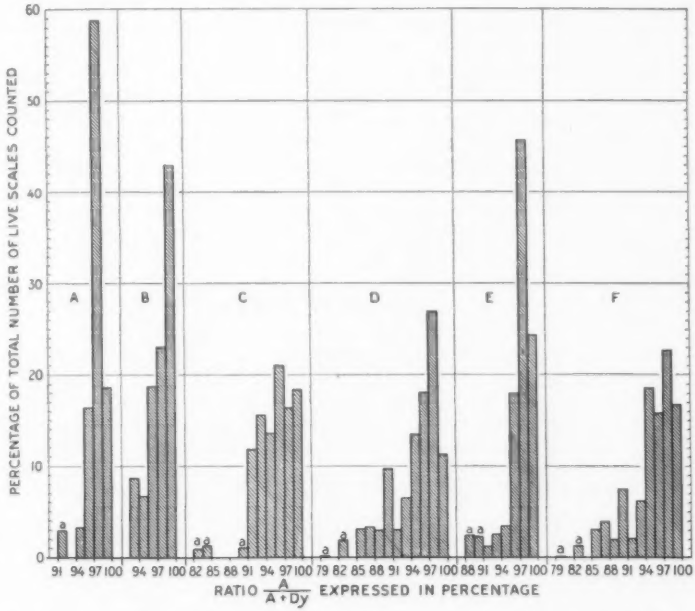

FIGURE 2.—Comparison of variability of the ratio of living scales to living plus dying scales $\left(\frac{A}{A+Dy}\right)$ from counts on fruits with the following factors involved: Grove from which collected, period of collection, whether stored or not, and, if so, the storage temperature. The frequency distributions are represented by the following groups in Table 1: A, Group 1; B, Group 2; C, Group 7; D, Group 8; E, Groups 1 2, 3, and 6; F, Groups 8, 9, and 10. *a* represents the count from a single sample

to 100 per cent. In D there is some concentration of scales in the interval 97 to 98.5 per cent, but the remainder are well distributed in the next higher and in the next eight lower intervals. In E the distribution resembles that of A, but with a less marked concentration in the interval 97 to 98.5 per cent, and a correspondingly larger percentage in each of the other intervals on either side of it. In F the general range of distribution is like that of D, but more evenly distributed in the four intervals between 94 and 100 per cent. The percentage values of one to three intervals in each of the frequency distributions except one appear to be more or less aberrant; that is,

these values are larger than would be expected from the positions of their intervals, which also may be separated from the others by blank intervals. This state of affairs may be due, at least in part, to the fact that these apparent aberrant values are derived from counts on single samples.

METHOD OF ELIMINATING NATURAL MORTALITY IN TESTS ON FUMIGATED SPECIMENS

Thus far only the natural mortality on untreated checks has been considered. In order to use these results in experimental tests, the fumigated scales must be counted after all those killed by treatment are distinguishable from living survivors by the criteria already presented, but before they have dried out enough to be confused with those that died before treatment. When fruits were stored promptly after treatment with hydrocyanic acid gas in the controlled rooms of the lowest (13° C. or 55.4° F.) and highest temperatures (26° C. or 78.8° F.) in which approximately 78 and 72 per cent relative humidities were maintained, respectively, all dying scales in the three periods could be distinguished four days afterwards. Counts could be continued with certainty for a week in the 26° C. environment and for two weeks in the 13° C. environment.

To eliminate the natural mortality in tests on fumigated samples counted in this way, the procedure is simply to multiply the number of living plus dying scales on the treated fruits by the percentage mortality on the corresponding checks as determined by the methods given here. This gives the number of individuals that are considered to be naturally dying. On subtracting this number from the number counted as dying, the number of scales actually killed by treatment is obtained, which is then compared with the number of survivors to secure the correct percentage of mortality due to the treatment. Two examples are given in Table 4 to illustrate the elimination of natural mortality from treated samples by means of the ratios $\frac{A}{A+Dy}$ and $\frac{A}{A+Dy+Dr}$ of the checks. The data for the checks, besides being presented collectively (records 7 and 13) for each treatment concerned, are given individually to bring out the variability in the numbers of scales involved and in the values for the ratios. The net mortality for each set of treated samples is calculated from the collective ratio values (records 8 and 14) and also from the extreme ratio values (records 9 and 10, 15 and 16). It is observed that in both sets the collective net mortality values obtained from the ratio $\frac{A}{A+Dy}$ are lower, and the extremes show distinctly less variability, than those obtained from the ratio $\frac{A}{A+Dy+Dr}$.

TABLE 4.—*Estimation of percentage of scales killed by hydrocyanic acid gas treatments using the ratio of dying scales to living plus dying scales* $\left(\frac{Dy}{A+Dy}\right)$ *and the ratio of living scales to the total number of scales* $\left(\frac{A}{A+Dy+Dr}\right)$ *for the elimination of natural mortality*

EXAMPLE 1

Record No.	Fruits used	Hydrocyanic acid schedule	Date treated	Date collected	Storage temperature	Counted after collection	Scales				Ratios		
							A	Dy	Dr	$A+Dy+Dr$	$\frac{A}{A+Dy}$	$\frac{Dy}{A+Dy}$	$\frac{A}{A+Dy+Dr}$
	Number				° *C.*	*Days*	*Number*	*Number*	*Number*	*Number*	*Per cent*	*Per cent*	*Per cent*
1	1	Check		Jan. 13, 1931	10	3	80	2	67	149	97.6	2.4	53.7
2						9	23	0	3	26	100.0	0	88.5
3						9	73	2	19	95	96.1	3.9	76.8
4						9	150	3	156	309	98.0	2.0	48.5
5						9	94	0	36	130	100.0	0	72.3
6						9	96	5	53	154	95.0	5.0	62.3
7	Total or weighted mean						516	13	334	863	97.5	2.5	59.8
8	7	110 per cent	Jan. 12, 1931	Jan. 13, 1931	10	11–14	43	239	390	672	15.2	84.8	6.4
9													
10													

Record No.	Fruits used	Hydrocyanic acid schedule	Date treated	Date collected	Mortality by writers' method					Mortality by usual method				
					$A+Dy$	$\frac{Dy}{A+Dy}$ of check	Dying naturally	Killed by treatment		$\frac{A}{A+Dy+Dr}$ of check	Survivors		Killed by treatment	
											Natural	Observed		
	Number				*Number*	*Per cent*	*Number*	*Number*	*Per cent*	*Per cent*	*Number*	*Number*	*Number*	*Per cent*
8	7	110 per cent	Jan. 12, 1931	Jan. 13, 1931	282	2.5	7.1	231.9	84.36	59.8	401.9	43	358.9	89.30
9					282	0	0	239.0	84.75	88.5	594.7	43	551.7	92.77
10					282	5.0	14.1	224.9	83.95	48.5	325.9	43	282.9	86.81

EXAMPLE 2

Record No.	Fruits used	Hydrocyanic acid schedule	Date treated	Date collected	Storage temperature	Counted after collection	Scales				Ratios		
							A	*Dy*	*Dr*	*A+Dy+Dr*	$\frac{A}{A+Dy}$	$\frac{Dy}{A+Dy}$	$\frac{A}{A+Dy+Dr}$
	Number				° *C.*	*Days*	*Number*	*Number*	*Number*	*Number*	*Per cent*	*Per cent*	*Per cent*
11	1	Check		Dec. 6, 1930	10	12	33	1	44	78	97.1	2.9	42.3
12							115	2	28	145	98.3	1.7	79.3
13	Total or weighted mean						148	3	72	223	98.0	2.0	66.4
14	8	133 per cent	Dec. 2–5, 1930	Dec. 3–6, 1930	10	9–12	27	775	442	1,244	3.4	96.6	2.2
15													
16													

Record No.	Fruits used	Hydrocyanic acid schadule	Date treated	Date collected	Mortality by writers' method					Mortality by usual method				
					A+Dy	$\frac{Dy}{A+Dy}$ of check	Dying naturally	Killed by treatment		$\frac{A}{A+Dy+Dr}$ of check	Survivors		Killed by treatment	
											Natural	Observed		
	Number				*Number*	*Per cent*	*Number*	*Number*	*Per cent*	*Per cent*	*Number*	*Number*	*Number*	*Per cent*
14	8	133 per cent	Dec. 2–5, 1930	Dec. 3–6, 1930	802	2.0	16.0	759.0	96.56	66.4	826.0	27	799.0	96.73
15					802	1.7	13.6	761.4	96.57	79.3	986.5	27	959.5	97.26
16					802	2.9	23.3	751.7	96.53	42.3	526.2	27	499.2	94.87

If it proves impossible to make adequate counts on natural mortality for use as given here, there is sufficiently small variation in the results obtained to justify an approximation. For female red scales from the fully developed second stage to the mature adult stage on lemons under field conditions similar to those at Whittier, Calif., a natural mortality of 4 per cent, exclusive of dried individuals, may be assumed. Though this figure is not based upon a full year's record, the work covers six months and includes the winter and spring seasons.

SUMMARY

In tests of hydrocyanic acid gas as a fumigant against the California red scale (*Chrysomphalus aurantii* Mask.), natural mortality as ordinarily considered varies so widely that better means of estimating it have been sought. By using lemon fruits exclusively and the three later stages in the development of the female only—that is, the fully developed second stage, the gray adult, and the mature adult—qualitative tests based on such factors as changes in body color and conformation have not given a satisfactory separation between natural mortality and mortality produced by treatment, which are mixed on treated samples, for any of these three periods. It has been found, however, that in each of the three periods natural mortality bears nearly a constant ratio to the number of living plus dying scales on untreated samples irrespective of a large number of varying factors ordinarily encountered in practice.

When counted at the proper time after treatment, practically all scales killed by treatment and those dying naturally can be distinguished, chiefly by changes in color and in viscosity of body contents. By using the relatively stable values obtained to express natural mortality, this factor can be eliminated from such counts on treated samples and a considerably higher degree of accuracy can be obtained than is possible by the old method of estimating the natural mortality from the ratio of living scales to total scale count.

This study indicates that it may be possible to determine constants for the correction of natural mortality in the California red scale, or in other insects, without separate counts for every experiment.

THE POSSIBLE EFFECT OF HYDROGEN-ION CONCENTRATION ON THE ABSORPTION OF POTASSIUM AND PHOSPHORUS BY WHEAT PLANTS UNDER FIELD CONDITIONS[1]

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INTRODUCTION

While studying the factors affecting the absorption of mineral elements by plants, the writer showed that the hydrogen-ion concentration of the medium affects the absorption of potassium and phosphorus by wheat seedlings grown in water cultures. (*4*) [2] More phosphorus was absorbed from potassium phosphate solutions with initial pH values of 5.0 and below than from those with pH values of 6.0 and 7.0. The results for potassium were not so definite as those for phosphorus. In several cases the absorption of potassium by the wheat seedlings was higher from the nearly neutral than from the acid solutions (pH 5.0 and below); in other cases the results were not consistent.

The concentrations of potassium phosphate used in these experiments were, for reasons explained in the article referred to, relatively high, but in concentrations as low as 14 parts per million of potassium and 12 parts of phosphorus (50 parts per million of monopotassium phosphate) the general character of the results was the same. From solutions of the lower concentrations (beginning with 500 parts per million of monopotassium phosphate), the absorption of potassium by the seedlings was practically always higher when the initial hydrogen-ion concentration of the solutions had a pH value of 7.0 than when it was 4.9. In view of the fact that potassium phosphate salts are all soluble it has been concluded that this effect of hydrogen-ion concentration on the absorption of phosphorus and potassium is physiological in nature.

EXPERIMENTAL METHODS AND DATA

It is frequently difficult to corroborate under field conditions, where a large number of known and unknown factors are dealt with, the results obtained with water cultures, where the conditions are practically controlled. Although it is true that wheat is being raised on soils with a considerable range of reaction, comparatively few chemical analyses of such crops are available, because experiment stations and other research institutions experimenting with wheat have been interested primarily in yield problems. Moreover, chemical analyses of cereals, when they are available, are largely limited to the grain, the composition of which fluctuates between narrower limits and is less subject to the influence of environment than the

[1] Received for publication July 22, 1932; issued April, 1933.
[2] Reference is made by number (italic) to Literature Cited, p, 452.

straw which is the vegetative part of the plant. For this reason the available data on the chemical composition of wheat are insufficient to throw light on the effect of soil reaction on the absorption and retention of the mineral elements by this plant.

In connection with an investigation on the relation of the lignin content to lodging of wheat (*5*), the writer was able to obtain data bearing indirectly on the effect of soil reaction on the accumulation (absorption and retention) of potassium and phosphorus by the wheat plant under field conditions. The experiment comprised two plots at the Arlington Experiment Farm, Rosslyn, Va. One served as control; the other received in the spring sodium nitrate at the rate of 600 pounds per acre. Samples for analysis were cut from the two plots at frequent intervals up to maturity. The results for the potassium and phosphorus content of the entire plant in the early stages of growth and of the stalks alone in the later stages are given in Table 1.

TABLE 1.—*Effect of applied sodium nitrate on the potassium and phosphorus content of wheat plants cut at various stages of growth*

[Percentage analyses on air-dry basis]

Date of sampling	K_2O in straw from—		P_2O_5 in straw from—	
	Control plot	Plot treated with sodium nitrate	Control plot	Plot treated with sodium nitrate
Apr. 30	2.5	3.63	0.73	0.52
May 7	2.11	3.50	.65	.51
May 14		3.12		.45
May 21	1.61	2.85	.62	.41
May 28	1.37	2.82	.54	.39
June 4	1.41	2.72	.47	.35
June 11	1.25	2.14	.41	.29
June 18	1.02	1.87	.26	.22
June 25	.53	.77	.22	.21

Potassium was consistently higher in all samplings from the plot treated with sodium nitrate than in the corresponding samplings from the control plot. In the case of phosphorus the reverse was true, its content being in every case higher in the straw from the control plot than in that from the fertilized plot. The results of the field experiments are therefore analogous to those of the waterculture experiment mentioned previously.

DISCUSSION

Sodium nitrate, although it has a neutral reaction when dissolved in distilled water, is a physiologically alkaline fertilizer. The plants absorb or retain more of the nitrate anion than of the sodium cation, and as a result the reaction of the medium of growth moves toward alkalinity. It is a well-known fact that when a soil is repeatedly treated with sodium nitrate it becomes alkaline. Some of this effect undoubtedly takes place even with one application of sodium nitrate.

The distribution of plant food in the soil, especially applied plant food, is never perfectly uniform; it is concentrated at points, to which

the roots are drawn by chemotropism. Theoretically the reaction should be less acid or more alkaline at the concentration points of applied sodium nitrate than in the remainder of the soil. But this is difficult to prove in view of the fact that the reaction obtained on testing is the average reaction at the spot at which the sample was taken. Similarly, the actual concentration of the soil solution available to the plants is stronger than the apparent concentration obtained by analysis, which gives only the average concentration.

No tests for soil reaction were made in connection with this experiment, but the application of sodium nitrate was high (600 pounds per acre), and it is reasonable to assume that it affected the reaction of the soil at least at the points at which it was concentrated. The soil reaction of the section of the Arlington Experiment Farm on which the experiment was carried out has a pH value of about 6.0. A change from this reaction toward alkalinity might have affected slightly the solubility of the soil phosphates, but it could hardly be expected to have affected the solubility of the soil potassium. Furthermore, the complete similarity between these field results and those obtained in the water-culture experiment, in which the possibility of a solubility effect was entirely eliminated, would indicate that the differences in absorption of phosphorus and potassium by the wheat plants in the field experiment were due to the same physiological causes responsible for similar differences occurring in the water-culture experiment.[3]

The writer has suggested an explanation of the effect of hydrogen-ion concentration on the absorption of potassium and phosphorus by plants, which was based on the isoelectric relations of the ampholytes of the living cell (*4*). This hypothesis has been extended to include the accumulation of cations and anions in growing plants in general. It is assumed that there is a wide range in the isoelectric points of the plant ampholytes, allowing the occurrence of both electropositive and electronegative ampholytes within certain limits of hydrogen-ion concentration. This makes possible the simultaneous accumulation of cations and anions. A change in the reaction of the medium may modify, to some extent, the reaction within the plant cells and thus cause a shift of some of the electropositive ampholytes to the negative side and vice versa. This may cause an increased absorption of cations or anions, respectively, which is the explanation offered for the effect of hydrogen-ion concentration on the absorption of potassium and phosphorus by wheat plants in the parallel experiments with water cultures and under field conditions.

Briggs and Petrie (*1*) have subsequently suggested exactly the same hypothesis to explain the simultaneous accumulation of cations and anions in the living cell. On the other hand, Hoagland, Davis, and Hibbard (*6, p. 484*) oppose this hypothesis stating that—

> It would be difficult to assume that individual proteins are available of such widely different isoelectric points as to permit the simultaneous formation of both cation and anion compounds at the pH values involved.

No reason is given why it is difficult to make such an assumption.

[3] If this explanation is correct ammonium sulphate, which shifts the soil reaction in the opposite direction from that caused by sodium nitrate, when applied under the conditions of the experiment reported in this article, would have resulted in an increase of the phosphorus content and in a decrease of the potassium content of the plant. However, the experiment was carried out with a different end in view, and the writer had no opportunity to repeat it with parallel applications of sodium nitrate and ammonium sulphate.

Our knowledge of the properties of proteins, presumably the principal ampholytes in plant tissues, is limited to isolated individual proteins which have been subjected to strenuous treatment in the process of isolation. Knowledge of the behavior of proteins in vivo is so far lacking. The same is true with regard to the available data on the hydrogen-ion concentration values of the cell sap, which are average values of sap expressed from dead or injured cells. The hydrogen-ion concentration of the sap in vivo may be entirely different. But even our limited knowledge of isolated proteins shows that proteins with quite divergent isoelectric points are present in the same plant tissue. Csonka, Murphy, and Jones (*3*), working with purified proteins from different sources, largely seeds, showed that the isoelectric points of the albumins are found at pH 4.0–5.0, whereas those of the prolamins are found at hydrogen-ion concentrations ranging from pH 6.0 to 6.5. Cohn, Gross, and Johnson (*2*) have found in potato and carrot tissue two proteins with isoelectric points at pH 4.0 and 8.0, respectively. It would seem that such differences are wide enough.[4]

SUMMARY

Wheat was grown on two field plots, one having received a relatively large application of sodium nitrate, the other serving as a control. Samples for analysis were cut at frequent intervals up to maturity.

The results for the potassium and phosphorus content of the entire plant in the early stages of growth, and of the stalks alone in the later stages, show that potassium was consistently higher in all samplings from the plot treated with sodium nitrate than in the corresponding samplings from the control plot, whereas phosphorus was higher in the samplings from the control than in those from the sodium nitrate plot.

These results are analogous to previously reported results from water cultures with controlled hydrogen-ion concentration, and are explained by a possible change in the hydrogen-ion concentration of the soil caused by the application of sodium nitrate which is a physiologically alkaline fertilizer.

A previously advanced hypothesis explaining the accumulation of cations and anions in growing plants based on the isoelectric relations of the ampholytes of the living cell is further discussed.

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[4] Since this article was prepared for publication there has appeared in Soil Science an article by Walter Thomas on The Reciprocal Effects of Nitrogen, Phosphorus and Potassium as Related to the Absorption of these Elements by Plants (7). In this otherwise full review the author, while discussing the effect of hydrogen-ion concentration of the medium of growth on the absorption of cations and anions by plants, failed to mention the hypothesis advanced by the present writer, also by Briggs and Petrie, explaining these phenomena on the basis of the isoelectric relations of the ampholytes of the living cell. Thomas seems to accept the hypothesis of Höber based on the electrostatic attraction caused by potential differences resulting from the activity of the root membrances, which varies with the reaction of the substrate. This explanation would have been adequate if the preferential accumulation of the mineral elements in the living cell were due entirely to preferential absorption. The generally accepted view, however, is that the accumulation of the mineral elements in living tissues is due to preferential retention rather than preferential absorption.

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GERMINATION OF THE SEED OF FARM CROPS IN COLORADO AFTER STORAGE FOR VARIOUS PERIODS OF YEARS [1]

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INTRODUCTION

Valuable data have been collected by various workers on the germination of seeds that have been stored a definite number of years. It is generally believed that in humid climates the seeds of farm crops lose their vitality within a few years. Under arid conditions little is known of the vitality of seeds after storage for several years. The study reported in this paper was begun with the idea of determining the viability of seeds of certain farm crops after storage for various periods.

There are very definite beliefs in various localities in regard to longevity of seeds, concerning seeds in general, and also a few particular seeds. The common belief in India (*8*)[2] is that seeds more than a year old are of no value for planting. Onion seeds are very generally considered of value only two or three years by both the seed trade and onion growers. In connection with the labeling of seeds in accordance with pure-seed laws, it is deemed advisable to retest seeds each year because germination frequently decreases materially from year to year.

WORK OF PREVIOUS INVESTIGATORS

That seeds in sealed bottles even in India retain their viability more than one year is brought out by Sonavne (*8*) in a report on a 4-year storage experiment. Wheat in 1922 showed a germination of 97.6 per cent and in 1926, 98.1 per cent. Corn during the same four years deteriorated in germinating ability from 96 per cent to 86 per cent.

Eastham (*4*) and Sifton (*7*) report practically no loss in vitality of oats stored in closed boxes for 10 years. Burgerstein (*1*), who experimented with seeds in a closed drawer, found that barley and oats deteriorated practically none in 10 years. He found that wheat deteriorated approximately 12 per cent and rye is reduced to 2 per cent in germination. Haberlandt (*6*) reports that barley loses one-half its germination ability in 4 years, corn and oats one-half in 6 years, wheat practically all in 6 years, and rye all in 3 years. Welton (*10*) working with seeds in bottles states that in 6 years oats and wheat are reduced to a germination of approximately 20 per cent, barley to 6 per cent, and corn to 49 per cent.

Crocker and Groves (*2*) and Groves (*5*), basing their experiments on the idea that effects of temperature are similar to aging at room temperature and that the time-temperature coefficient for coagulation of proteins applies, predict the life duration of wheat as follows:

[1] Received for publication July 22, 1932; issued April, 1933.
[2] Reference is made by number (italic) to Literature Cited, p. 462.

Seed containing 17.5 per cent moisture held at 50° C. would survive for 17.1 days, and seeds of the same wheat at 25° would live 21.1 years. Seed containing only 9 per cent of moisture would when kept at 50° live 53 days and when held at 25° would live 37 years. Duvel (*3*) in an experiment to show what would happen to seeds in storage in various parts of the country concluded that in approximately 250 days there was little change if the air was dry and the temperature range normal, but that when various vegetable and flower seeds were stored in moist air there was considerable deterioration at 32° to 37°.

EXPERIMENTAL METHOD AND RESULTS

The first tests were made on the 1920 crops. The grains were threshed, cleaned, and stored in 100-pound sacks, which were then placed in an unheated room and kept there during the entire period of the test. Samples were taken in February by mixing the grain thoroughly and drawing off a portion with a small scoop. Germination tests were made in February of each year. Crops were saved each year when grown and placed in the storage room. Only perfect seeds were used for germination, the broken and damaged seeds being discarded. The crops used were the common standard varieties of cereals shown in Table 1.

TABLE 1.—*Varieties of seed studied and years in which they were grown*

Crop	Variety	Plus mark signifies crop was grown in year indicated									
		1920	1921	1922	1923	1924	1925	1926	1927	1928	1929
Spring wheat	Marquis		+	+	+	+	+	+	+		+
Winter wheat	Kanred		+			+					+
Spring wheat	Deflance			+		+		+	+		
Durum wheat	Kubanka		+	+							+
	Colorado 37	+		+	+	+	+	+	+	+	+
	Great Dakota			+	+						
Oats	Swedish Victory	+		+							
	White Russian	+									
	Gold Rain						+	+			
	Nebraska 21			+		+	+	+	+	+	+
	Nepal	+	+	+	+	+	+				
	Success	+	+	+							
	Colsess			+	+	+	+	+	+	+	+
Barley	Coast	+	+		+	+	+				
	Hanna	+		+							
	Gold	+									
	Moister						+	+	+		
Winter rye	Rosen			+	+	+				+	
Soybean	Wisconsin Black				+	+					
Sorghum	Black Amber						+	+	+		
Corn	Yellow dent		+	+	+	+					

The average rainfall and relative humidity "½ (7A + 7P)"[3] in percentage of saturation is given in Table 2. These data were taken from Bulletin 340 (*9, Tables 17 and 21*)[4] of the Colorado Agricultural Experiment Station.

[3] To obtain the relative humidity, readings were made twice daily at 7 a. m. and 7 p. m., and the average of the two readings taken as the mean (*9, p. 11,* and *Table 17*).

[4] Data for 1928, 1929, and 1930 were obtained directly from Mr. Trimble.

TABLE 2.—*Rainfall and relative humidity during period covered by experiments*

Year	Average annual precipitation	Average annual relative humidity	Year	Average annual precipitation	Average annual relative humidity	Year	Average annual precipitation	Average annual relative humidity
	Inches	*Per cent*		*Inches*	*Per cent*		*Inches*	*Per cent*
1920	11.65	70.2	1924	10.64	64.8	1928	13.54	65.3
1921	14.83	66.2	1925	14.50	64.3	1929	13.73	66.8
1922	9.98	67.6	1926	13.56	66.4	1930	15.17	64.8
1923	27.57	71.6	1927	15.77	67.2			

There appears to be no connection between the average annual relative humidity and the original germination percentage. Humidities for all years were low. The actual percentage of moisture in the seed samples was determined for the 1929 crop and was found to range between 9.5 and 11.4 per cent. No tests were made on the other crops.

The germination tests made the year the seeds were harvested ranged from 93 to 98.5 per cent for Marquis, 84 to 90 per cent for Kubanka, 93 to 95 per cent for Kanred wheat. (Table 3.) Barley and rye showed a greater variation from year to year and for different varieties, while the percentage of germination of oats did not differ greatly from that of wheat.

TABLE 3.—*Germination percentages for various seeds harvested in different years*

Crop	1920	1921	1922	1923	1924	1925	1926	1927	1928	1929
Wheat:										
Marquis	.....	98.0	97.5	93.0	93.5	97.5	95.5	98.5	.....	97.0
Kubanka	.....	88.0	90.0	.....	.....	.....	.....	.....	.....	84.0
Kanred	.....	95.0	.....	.....	94.0	.....	.....	.....	.....	93.0
Barley:										
Nepal	87.0	95.5	95.0	73.0	69.0	84.0	.....	.....	.....	.....
Colsess	.....	.....	99.5	98.5	97.0	97.0	94.0	97.0	97.0	99.5
Coast	97.0	94.0	.....	98.5	92.0	96.5	.....	.....	.....	.....
Moister	.....	.....	.....	.....	.....	98.5	96.0	97.0	.....	.....
Oats:										
Colorado 37	98.0	.....	99.0	99.0	98.0	97.0	97.0	95.0	98.5	98.0
Nebraska 21	.....	.....	97.0	97.5	95.5	94.0	98.0	.....	92.0	.....
Miscellaneous crops:										
Rye	.....	.....	98.0	83.5	84.0	.....	.....	.....	.....	.....
Soybeans	.....	.....	.....	93.5	.....	.....	.....	.....	.....	.....
Sorghum	.....	.....	.....	.....	.....	.....	.....	92.0	.....	.....
Corn	.....	.....	.....	85.0	98.0	.....	.....	.....	.....	.....

The general trend seems to indicate that oats, covered barley, Marquis and Kanred wheat have a high percentage of germination the first year, while Kubanka wheat, Nepal barley (naked), and rye show a comparatively low germination percentage the first year.

A study of the germination of the various seeds as a percentage of the original germination shows that when all varieties of the same crop are averaged there is a slow decline in the germination percentage for the first 10-year period. Table 4 gives the average percentage of germination for all seeds studied, as well as the average for each variety, and the number of years in the test period.

WHEAT VARIETIES

The table gives the average germination for all wheat seed as well as the average for each variety. Three varieties of *Triticum vulgare* and one variety of *T. durum* were tested. The commercial classes are represented by three varieties. Marquis is a hard red spring wheat. The germination percentage of this variety falls off 8.7 per cent in 10 years. Kanred, a hard red winter wheat, shows a similar drop. Defiance, a soft white wheat, shows the same general trend but drops off less than Marquis.

TABLE 4.—*Germination percentages of wheat, barley, oats, and miscellaneous seeds grown in Colorado when the original test is valued at 100 per cent and the other tests are calculated as percentage of the first year's test*

Crop and variety of seed	Percentage germination and number of crops tested in indicated number of years after harvesting									
	1	2	3	4	5	6	7	8	9	10
Wheat:										
Marquis—										
Germination.....per cent	100	99.8	98.1	97.5	96.7	95.1	95.9	93.1	93.4	91.3
Crops tested.....number	9	8	7	7	6	5	4	3	2	1
Defiance—										
Germination.....per cent	100	98.4	99.3	98.2	96.7	97.9	96.2	96.4	94.3	.....
Crops tested.....number	4	4	4	4	3	2	2	1	1	.....
Kanred—										
Germination.....per cent	100	102.5	98.2	100.5	97.8	96.9	94.5	93.7	90.1	98.4
Crops tested.....number	3	3	2	2	2	2	2	1	1	1
Kubanka—										
Germination.....per cent	100	100.7	97.8	97.5	95.0	98.7	94.2	92.5	84.9	89.8
Crops tested.....number	3	3	2	2	2	2	2	2	2	1
Average germination of all wheats.....per cent	100	100	98.4	98.1	96.6	96.6	95.3	93.4	90.2	93.2
Total number of crops tested	19	18	15	15	13	11	10	7	6	3
Standard error	±0.93	±0.66	±0.80	±1.15	±1.19	±1.44	±1.15	±1.15	±2.27	.....
Barley:										
Nepal—										
Germination.....per cent	100	99.3	89.8	86.2	88.3	85.9	92.3	83.2	81.3	75.8
Crops tested.....number	6	6	4	6	6	6	5	4	3	2
Success—										
Germination.....per cent	100	97.8	95.3	97.0	98.5	94.1	96.4	94.9	89.8	89.1
Crops tested.....number	3	3	1	2	2	3	3	3	3	2
Colsess—										
Germination.....per cent	100	98.5	97.6	98.7	96.8	95.2	93.5	96.0	93.9	.....
Crops tested.....number	8	8	7	6	5	4	3	2	1	.....
Coast—										
Germination.....per cent	100	100.5	100	99.6	98.6	98.8	98.3	96.9	98.5	101.4
Crops tested.....number	5	5	5	5	5	5	4	3	2	2
Hanna—										
Germination.....per cent	100	97.3	104.3	99.3	100.4	94.9	90.2	90.8	90.7	86.7
Crops tested.....number	2	1	2	2	2	2	2	2	2	1
Gold—										
Germination.....per cent	100	97.4	99.5	98.4	93.7	87.4	81.0	79.1	78.0	68.1
Crops tested.....number	1	1	1	1	1	1	1	1	1	1
Moister—										
Germination.....per cent	100	99.8	99.3	99.6	94.4	95.9	.....	.....	.....	.....
Crops tested.....number	3	3	3	3	2	1	.....	.....	.....	.....
Average germination of all barley.....per cent	100	99.0	97.6	95.9	95.1	93.0	93.7	90.7	88.6	85.9
Total number of barley crops tested	28	27	23	25	23	22	18	15	12	8
Standard error	±1.40	±0.63	±1.04	±1.56	±1.48	±1.66	±2.95	±1.80	±2.11	.....
Average germination of naked barley.....per cent	100	99.3	89.8	86.2	88.3	85.9	92.3	83.2	81.3	75.8
Total number of crops of naked barley tested	6	6	4	6	6	6	5	4	3	2
Average germination of 6-row hooded covered barley.per cent	100	98.3	97.3	98.2	97.2	94.7	94.9	95.3	90.8	89.1
Total number of crops of 6-row hooded covered barley tested	11	11	8	8	7	7	6	5	4	2
Average germination of 6-row covered awned barley.per cent	100	100.2	99.8	99.6	97.4	98.3	98.3	96.9	98.5	101.4
Total number of crops of 6-row covered awned barley tested	8	8	8	8	7	6	4	3	2	2
Average germination of 2-row covered awned barley.per cent	100	97.4	102.6	99.0	98.1	92.4	90.5	86.9	77.4	79.8
Total number of crops of 2-row covered awned barley tested	3	2	3	3	3	3	3	3	2	2

TABLE 4.—*Germination percentage of wheat, barley, oats, and miscellaneous seeds grown in Colorado when the original test is valued at 100 per cent and the other tests are calculated as percentage of the first year's test*—Continued.

Crop and variety of seed	Percentage germination and number of crops tested in indicated number of years after harvesting									
	1	2	3	4	5	6	7	8	9	10
Oats:										
Colorado 37—										
Germination.....per cent	100	101.4	100.8	97.8	95.5	95.3	95.3	94.6	95.0	91.9
Crops tested.....number	9	8	8	7	6	5	4	3	2	1
Great Dakota—										
Germination.....per cent	100	97.8	97.8	95.7	96.5	94.8	93.0	95.5	89.4	-----
Crops tested.....number	2	2	2	2	2	2	2	2	2	-----
Swedish Victory—										
Germination.....per cent	100	95.0	99.3	97.8	93.5	92.7	94.5	92.7	91.0	81.8
Crops tested.....number	2	1	2	2	2	2	2	2	2	1
White Russian *—										
Germination.....per cent	-----	98.5	-----	97.0	98.0	95.0	93.5	94.5	97.5	88.0
Crops tested.....number	1	1	-----	1	1	1	1	1	1	1
Gold Rain—										
Germination.....per cent	100	99.5	99.9	97.1	98.5	91.6	-----	-----	-----	-----
Crops tested.....number	2	2	2	2	2	1	-----	-----	-----	-----
Nebraska 21—										
Germination.....per cent	100	100.6	97.7	97.3	95.7	95.7	98.5	88.1	92.8	-----
Crops tested.....number	7	6	6	5	3	3	2	1	1	-----
Average germination of all oats .....per cent	100	100.1	99.4	97.3	95.9	94.7	95.1	93.7	92.6	87.2
Crops tested.....number	23	20	20	19	16	14	11	9	8	3
Standard error.....	±0.44	±0.97	±0.91	±0.68	±0.72	±0.68	±0.87	±0.94	±2.65	-----
Average germination of midseason oats.....per cent	100	100.2	100.0	97.4	95.3	94.6	94.5	94.3	92.3	86.9
Total number of crops tested...	13	11	12	11	10	9	8	7	5	2
Rosen rye:										
Germination.....per cent	100	98.1	98.4	90.6	90.1	89.6	79.8	79.1	52.0	-----
Crops tested.....number	4	3	3	3	3	3	3	2	1	-----
Wisconsin black soybeans:										
Germination.....per cent	100	101.1	93.0	86.6	90.1	83.3	61.2	48.1	-----	-----
Crops tested.....number	2	2	2	2	2	2	2	1	-----	-----
Black Amber sorghum:										
Germination.....per cent	100	117.0	119.0	115.8	104.4	97.9	-----	-----	-----	-----
Crop tested.....number	3	3	3	3	2	1	-----	-----	-----	-----
Corn:										
Germination.....per cent	92	90	97	97	87	88.4	86.9	80.2	79	-----
Crops tested.....number	2	1	2	2	3	5	6	5	2	-----

* Actual percentages, because of lack of test the first year.

Kubanka, a durum wheat, drops off slightly for eight years and then makes a quick drop. However, further tests may smooth out the discrepancy.

The average of all wheats tested shows a gradual drop for a 9-year period. There is a slight rise in the tenth year, but as only three years are represented in this test, less reliance can be placed on it. The standard errors were worked for all years up to the ninth year and show uniformity, with the exception of the ninth year which shows a wider variation, due, possibly, to the low number of years in the test. Wheat seed stored in a dry unheated room lose about 7 per cent of their germination in a 10-year period under Colorado conditions.

The actual germination of all wheat varieties tested is given in Table 5. This table represents the types of data obtained with the barley and oats studied in the germination experiment.

TABLE 5.—*Germination percentage of different wheat varieties grown at Fort Collins, Colo., 1921–1930*

Variety	Year grown	Percentage of germination in indicated number of years after harvesting									
		1	2	3	4	5	6	7	8	9	10
Marquis	1921	98.0	97.0	96.0	92.5	93.5	92.5	94.5	88.5	94.0	89.5
	1922	97.5	97.5	99.0	98.5	97.0	97.5	93.0	94.0	88.5	.....
	1923	93.0	94.5	83.0	81.5	86.5	79.0	84.0	86.0	.....	.....
	1924	93.5	96.5	96.5	93.5	95.0	92.5	95.0	.....	.....	.....
	1925	97.5	93.5	94.5	87.5	88.5	95.0	.....	.....	.....	.....
	1926	95.5	96.5	93.0	95.0	95.5	.....	.....	.....	.....	.....
	1927	98.5	96.5	99.0	98.5	.....	.....	.....	.....	.....	.....
	1928	97.0	96.5	.....	.....	.....	.....	.....	.....	.....	.....
	1930	97.0	.....	.....	.....	.....	.....	.....	.....	.....	.....
Kanred	1921	95.0	97.0	94.0	95.5	93.5	92.5	88.0	89.0	86.0	93.5
	1924	94.0	95.0	91.5	94.5	91.0	90.5	90.5	.....	.....	.....
	1929	93.0	97.0	.....	.....	.....	.....	.....	.....	.....	.....
Defiance	1922	96.0	95.0	96.0	94.0	95.0	94.0	95.0	92.5	90.5	.....
	1924	89.0	91.0	88.5	91.5	89.5	87.0	83.0	.....	.....	.....
	1926	90.5	86.0	90.5	85.5	82.0	.....	.....	.....	.....	.....
	1927	98.5	96.0	96.5	96.0	.....	.....	.....	.....	.....	.....
Kubanka	1921	88.0	92.5	86.5	89.5	88.5	91.5	86.0	84.5	79.0	79.0
	1922	90.0	86.5	87.5	84.0	80.5	84.0	81.5	80.0	72.0	.....
	1929	84.0	84.5	.....	.....	.....	.....	.....	.....	.....	.....

BARLEY VARIETIES

The results of the barley varieties tested are also shown in Table 4. The 6-row hooded covered barleys decreased in germination about 10 per cent in 10 years. However, Coast showed a drop of only about 3 per cent. Nepal, a hull-less barley, dropped off about 20 to 25 per cent, the decrease starting about the third year. Two varieties of 2-row barley were tested, and they showed a more marked drop than did the 6-row barleys.

When the average of all barleys grown is considered, there is a gradual drop in percentage germination with indications of a greater drop after the ninth year. The total drop equals 14.1 per cent in 10 years, which is slightly greater than that of all wheat varieties.

If only 6-row hooded covered barleys are considered, there is slightly less falling off than with wheat. There seems to be a greater tendency for naked and 2-row barleys to lose vitality than for 6-row covered awned varieties. The standard error is slightly higher than that for wheat. However, it still shows a significant difference between the first and ninth year tests.

OAT VARIETIES

Both midseason and early oats were used in this test. Only varieties of *Avena sativa* and *A. sativa orientalis* were used in the experiment. Colorado No. 37, a high yielding oat of the midseason type, decreased in germination 8.1 per cent in 10 years. (Table 4.) Great Dakota showed a similar tendency. Swedish Victory decreased to 93.5 per cent in the fifth year and remained fairly uniform until the eighth year. A slight drop was noticed in the ninth year and a marked drop in the tenth year. Only two lots of this oat were used in the test, and the tenth year is represented by only one lot. White Russian, a side oat, decreased slightly until the eighth year, then increased in the ninth and decreased in the tenth year, dropping 12 per cent.

Nebraska 21, an early oat, shows a similar trend to the midseason oats, decreasing slightly each year, with slight variations, possibly due to sampling error.

When the average of all midseason oats tested is considered, there is a gradual decrease in germination for nine years, with a marked drop in the tenth year, when only two lots were tested. A very similar condition is found when all oats tested are considered.

OTHER SEEDS

Less complete data were obtained for other seeds. Rosen, a winter rye, decreased noticeably after the third year. Three lots of this variety were used in the test and by the seventh year, a drop of 20.2 per cent had been obtained. More data are necessary before final conclusions for the ninth and tenth years can be drawn.

Only one variety of soybeans was tested. Wisconsin Black, an early maturing bush type of bean, was used. This variety decreased in germination percentage after the fifth year and continued to drop rapidly after that period. Varying percentages of hard seeds were found in the soybeans tested. The number, however, fell off gradually until the fourth year, when all viable seeds germinated.

Black Amber, a forage sorghum adapted to northern Colorado, had lost very little of its viability after being stored for six years.

Several varieties of yellow dent corn were used. The test for corn is the most incomplete of the series. However, the data were included because of the increase of acreage of this crop in the past 10 years. Corn held its germination percentage well for a 4-year period. After this stage it decreased to a marked extent and again remained uniform until the seventh year, after which it again commenced to drop, losing 21 per cent of its germination by the ninth year.

The data for the last four kinds of seed are less conclusive than those for wheat, barley, and oats. However, they indicate that other farm seeds, such as winter rye, soybeans, sorghum, and corn stored in dry rooms may be used after five years with only a slight increase in the rate of seeding in order to obtain a good stand.

SUMMARY

Germination tests were made on the seed of various cereal crops adapted to Colorado conditions after being stored for varying periods of years in a dry room.

The germination percentage of wheat decreased about 7 per cent in 10 years.

The germination percentage of barley decreased about 14 per cent in 10 years. Two-row and naked barley decreased more than hulled 6-row barley.

The germination percentage of both midseason and early oats decreased about 13 per cent in 10 years.

Rosen rye and Wisconsin Black soybeans decreased 10 per cent in germination in 5 years. After the fifth year the germination percentage decreased rapidly.

Black Amber sorghum germinated well for six years, decreasing 2 per cent in this time.

Yellow dent corn germinated well for the first four years but decreased 13 per cent in the fifth year. There was a decrease of 20 per cent after eight years of storage.

Small-grain crops stored in a dry, unheated room, under the climatic conditions at Fort Collins, have still a high percentage of viable seed when 10 years old.

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THE RELATIVE DIGESTIBILITY BY RATS OF THE MILK OF FIVE BREEDS OF DAIRY CATTLE[1]

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INTRODUCTION

More than 40 per cent of the milk produced in the United States is consumed as fluid milk, while more than 30 per cent is used in the manufacture of butter. About 3 per cent of the milk is used in calf feeding. Much of the skim milk and buttermilk resulting from butter making is fed to livestock. The thoroughness with which the components of whole milk and also those of creamery by-products fed to livestock are digested, is a matter of economic importance.

Because of differences in the chemical composition and physical characteristics of the milk of the breeds of dairy cattle, a question naturally arises regarding the relative digestibility of the milk of these breeds. The greatest difference in chemical composition lies in the percentage fat content, but differences are found also in the percentages of protein, lactose, and ash (*21*).[4] Differences exist in the size of the fat globules and the rapidity with which they rise (*6, p. 143*).

Much interest in the relative digestibility of the milk of the breeds of dairy cattle has been aroused through the observations of digestive disturbances in artificially fed infants receiving a milk diet. Some of these disturbances seem to follow the ingestion of milk high in fat content, indicating a limited capacity of certain individuals for the digestion of fat. The work of Hill (*9*) has shown that the protein in the milk of some cows forms a "soft curd" while that in the milk of other cows forms a "hard curd." The soft-curd milk has been found of value for infants suffering from digestive troubles. Several experiments upon the completeness of digestibility of milk fat by adults show slight differences in the digestive coefficients. It seems evident, therefore, that several factors may be involved in the digestion of milk, particularly in the case of infants. It is clear that a thorough study of the digestibility of milk by infants would necessitate employing them as subjects.

The only phase of digestibility studied in the experiments reported in this paper is the completeness of digestibility of the components of milk. The investigation was prompted by the fact that the authors know of no animal experiments which would afford direct comparisons of the completeness of digestibility of the milk of the various dairy-cattle breeds. It is not assumed that the experiments have any bearing upon digestive troubles either of infants or of adults, or upon the time required for gastric digestion, or upon any phase of the

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[3] The analytical work involved in this investigation was done by R. H. Sifferd, under the direction of O. R. Overman of the Dairy Chemistry Division. Their assistance is gratefully acknowledged.
[4] Reference is made by number (italic) to Literature Cited, p. 471.

digestive process other than completeness of digestibility. In fact, since the digestibility of milk by infants appears to involve so many factors, the authors disclaim any attempt to apply the results of these experiments to the field of infant nutrition.

The use of albino rats naturally raises a question concerning the application of the results to other species. While the results of nutrition studies conducted with rats may not apply in toto to other species, it is believed that in experiments such as the present, in which comparative results were sought, the findings may be applied to a reasonable extent to the human adult and to animals of other species which normally consume milk, such as young calves and pigs. For example, should experiments with rats show pronounced differences in the digestibility of the milk of two breeds of dairy cattle, it is only reasonable to assume that about the same relative differences in digestibility would be shown by other species which normally consume milk, although the actual digestion coefficients secured with various species might differ considerably.

REVIEW OF THE LITERATURE

On account of the fact that the greatest difference in the chemical composition of the milk of the dairy-cattle breeds lies in the percentage fat content, the study of the digestibility of the fat was a point of special interest. The review of the literature, therefore, has been limited to experiments in which the completeness of digestibility of milk fat was determined. This is presented in condensed form in Table 1.

TABLE 1.—*Condensed review of the literature*

Investigator	Subject	Food	Coefficient of apparent digestibility		
			Fat	Protein	Dry substance
Rubner (*22*)	Human	Milk only	95.4	93.0	91.6
			94.4	92.3	90.6
			92.9	88.0	89.8
Do	do	Butter [a]	96.3		
			97.3		
Rubner (*23*)	do	do [a]	91.5		
Mayer (*18*)	do	do [a]	98.3		
			97.1		
Atwater (*2*)	do	do [a]	91.0		
Do (*3*)	do	Milk	97.0	88.1	
Do. (*4*)	do	Butter [a]	96.7		
Hultgren and Landergren (*16*)	do	do [a]	97.3		
			94.6		
Bryant (*5*)	do	Milk only	95.7	93.8	
Lührig (*15*)	do	Butter [a]	95.7		
Do. (*16*)	do	do [a]	96.9		
			97.2		
Kienzl (*15*)	do	do [a]	96.7		
Wibbens and Huizenga (*24*)	do	do [a]	97.3		
			96.5		
Doane and Price (*7*)	Dairy calves	Milk only	96.8	94.8	
			96.1	92.6	
Do. (*8*)	do	do	97.2	93.5	
Von Gerlach (*10*)	Human	Butter [a]	97.0		
Langworthy and Holmes (*12*)	do	do [a]	93.9		
Do. (*13*)	do	Cream [a]	92.7		
Do. (*14*)	do	Butter [a]	94.6		
Lundgren, Perander, and Putkonen (*17*)	do	do [a]	94.8		
Holmes (*10*)	do	do [a]	97.0		
			96.1		
Hughes and Cave (*11*)	Dairy calves	Milk [b]	98.5	95.7	
			99.0	94.8	

[a] As part of a simple mixed diet.

[b] Partially skimmed.

EXPERIMENTAL METHODS

It is assumed that the differences between the amounts of protein, fat, sugar, and total solids in the milk consumed and the amounts of these excreted in the feces represent the amounts digested. It is believed that determinations of digestibility made in this way are accurate even though products of endogenous origin are present in the feces. The amount of these products is undoubtedly closely related to the amount and character of the food ingested and may be considered one of the necessary costs of digestion. Because the method of calculation used in these studies makes no allowance for the presence of the endogenous substances in the feces, the term "apparent digestibility" is used.

The dairy breeds whose milk was compared were Jersey, Guernsey, Holstein-Friesian, Ayrshire, and Brown Swiss. The trials were conducted with albino rats. In all the trials except those with Brown Swiss milk, healthy rats from the department's stock colony were used. In order to keep them in good physical condition, they were fed the mixture used for the stock colony for a few days following each digestion trial. The studies with Brown Swiss milk were conducted in connection with a growth experiment in which the animals became anemic. These received fresh whole milk only from time of weaning, with no changes whatever in the manner of feeding.

The animals were confined individually in glass cages below which pans lined with heavy filter paper were placed to receive the excreta. The rods in the bottoms of these cages were spaced far enough apart so that the feces dropped through very readily and rarely lodged in the cage. In the digestion trials for protein, clean filter papers were placed in the pans daily; in trials for the other components, they were renewed on alternate days.

The digestion trials were 10 days in length. Each was preceded by a preliminary period of 3 to 5 days, during which the particular milk to be tested was fed. Milk was the only food given during the digestion trials. The amounts fed were adjusted during the preliminary periods to levels slightly below the maximum amounts which the animals would consume. Efforts were made to keep the daily intake for each animal constant throughout a 10-day trial. In most of the trials, paired feeding was practiced so that the animals of a pair consumed different kinds of milk in the same amounts. In these trials, two animals of the same sex and of approximately the same weight formed a pair.

The milk was secured from the university dairy barns daily. The containers were washed thoroughly each day with washing powder and then rinsed with a concentrated sulphuric acid-sodium dichromate cleaning solution. The milk was kept in a refrigerator and was never more than 24 to 28 hours old when fed. Even though unheated, it was always in excellent condition. Feedings were given twice daily. The containers used were glass sponge dishes, and after each feeding these dishes were carefully washed, rinsed with distilled water, and drained on a drying rack constructed of glass rods. The careful sanitary methods used are believed to have been responsible for the fact that practically no cases of milk souring occurred. The feed records, therefore, are uncomplicated by irregular or lowered intake of milk on account of its condition.

The Brown Swiss milk, as secured daily, consisted of the mid-portion of a single milking of an individual cow. The milk of three different Brown Swiss cows was used, but in no case was the milk of more than one of these cows used in any one digestion trial. About equal numbers of coefficients were secured with the milk of the three cows. The milk of the other breeds was taken daily from the mixed milk of a single complete milking of each of four cows. The milk was taken at a time when the cows were neither near the beginning nor the close of their lactation periods.

It is recognized that the number of cows employed is small. This procedure is believed to be justified in view of the fact that this study had for its chief object the discovery of any differences which may exist in the digestibility of the milk of the dairy-cattle breeds. It has been shown (*21*, *Table 6*) that the variations in the composition of the milk of individual cows within a breed are far greater than the differences between the mean values of the milk components of the various breeds. The use of samples of the mixed milk of a large number of cows of a breed for digestibility studies, therefore, would be likely to minimize and obscure any differences between breeds, while the use of samples of milk from individual cows or from small groups of cows would be more likely to demonstrate differences, should any exist.

Samples were taken from each lot of milk before feeding. Small amounts of formalin were added and the samples were kept in a refrigerator until analyzed. Analyses were made of composite samples.

The feces were removed from the collection pans daily. In the digestion trials for the determination of protein, the feces were transferred directly to stoppered Kjeldahl flasks containing 50 c c chemically pure sulphuric acid. When it was evident that some of the feces had been contaminated by urine, they were transferred, before being placed in the Kjeldahl flasks, to a sheet of clean filter paper laid over hardware cloth and washed by a stream of hot, slightly acidified, nitrogen-free water from a wash bottle. This procedure was changed in the last two trials. Trays made of hardware cloth having eight meshes to the inch were placed below the cages on top of the collection pans and the feces were transferred from the trays directly to the Kjeldahl flasks with no washing in any case. In the second of these last two trials, the rats were carefully clipped to prevent hair from being ingested or falling into the collection trays and adhering to the feces.

In the trials for the determination of fat, total solids, and sugar, the feces were transferred daily to paraffined paper cups, in which they were dried for two to three hours at a temperature of 60° to 70° C. They were then stored in glass jars in a refrigerator until analyzed.

The methods of analysis prescribed by the Association of Official Agricultural Chemists (*1*) were followed. Qualitative tests only for sugar were made, both Benedict's and Fehling's methods being used.

PRESENTATION OF RESULTS

A summary of the digestion coefficients secured is given in Tables 2, 3, and 4. Most of the detailed data secured with Brown Swiss milk and a few of those obtained with Holstein milk have been presented in previous papers (*19*, *20*).

TABLE 2.—*Comparative apparent digestibility of the fat of the milk of dairy cattle* [a]

	Summary of coefficients of apparent digestibility of milk fat				
	Ayrshire	Brown Swiss	Guernsey	Holstein-Friesian	Jersey
	99.1	99.6	98.6	98.9	97.1
	98.6	99.7	98.9	98.5	98.8
	98.4	99.6	97.4	98.6	98.5
	98.1	99.7	98.1	98.1	98.6
	99.3	98.8	99.2	98.7	98.4
	97.8	99.5	98.7	98.0	98.2
	98.9	99.4	98.4	98.7	98.9
	98.2	99.4	99.2	98.9	98.7
	98.7	99.4	98.7	98.4	98.7
	98.6	99.4		99.0	98.5
		99.4		95.5	
		99.3		96.5	
		99.6		98.2	
		99.3		98.8	
		99.5		98.5	
		99.7		98.2	
		99.5		98.3	
		99.6		97.6	
		99.2		97.9	
		99.0		99.0	
		99.5			
		98.0			
		99.4			
		98.8			
		98.8			
		98.3			
		97.9			
		98.2			
		98.1			
		98.4			
		98.1			
		98.4			
		98.9			
		98.7			
		98.4			
Mean	98.6 ±0.09	99.0 ±0.07	98.6 ±0.11	98.2 ±0.13	98.4 ±0.10
Standard deviation	.44± .07	.57± .05	.49± .08	.84± .09	.49± .07
Coefficient of variability	.44± .07	.58± .05	.50± .08	.86± .09	.49± .07

[a] Number of determinations. Ayrshire, 10; Brown Swiss, 34; Guernsey, 9; Holstein-Friesian, 20; Jersey, 10.

The fat of the milk of dairy cattle was found to be highly digestible. (Table 2.) When it is considered that extraction of feces with warm ether for removal of the fat almost certainly removes ether-soluble substances other than food fat residues, it seems reasonable to assume that there was very little if any undigested milk fat in the extracts. The conclusion naturally follows that the fat of fresh whole milk fed in these experiments was nearly all, if not entirely all, digested.

Practically no differences in the apparent digestibility of the milk fat of the five breeds were found. (Table 2.) The means of the coefficients for Ayrshire, Brown Swiss, Guernsey, and Jersey milk are practically the same. Moreover, the standard deviations and coefficients of variability are very similar. The mean of the coefficients for the fat of Holstein milk is slightly below that of the other breeds, caused in part by two unexplainably low coefficients, namely, 95.5 and 96.5. Such variations in results are likely to occur in biological investigations of this sort and no special significance should be attached to them. In fact, it is rarely possible to secure results of 10-day digestion trials which agree as closely as the 83 values shown in Table 2. With the exception of these two low coefficients, all coefficients for the five breeds are above 97, and only six others are below 98. If the two lowest coefficients for Holstein milk (95.5 and 96.5) be omitted, then the mean of the coefficients is 98.5 ± 0.06, a value which is nearly the same as the values for the other breeds.

TABLE 3.—*Comparative apparent digestibility of the total protein of the milk of dairy cattle* [a]

Summary of coefficients of apparent digestibility of milk protein				
Ayrshire	Brown Swiss	Guernsey	Holstein-Friesian	Jersey
a	*d*			
87.5	95.8	91.0	91.6	88.2
90.6	95.7	88.8	92.6	92.9
91.0	93.4	92.2	92.3	91.7
90.5	92.8	87.3	92.4	89.2
91.2	*e*	93.1	94.1	90.0
88.7	93.7	88.4	94.7	88.4
88.1	94.1	88.7	93.0	90.4
89.2	94.1	88.9	93.1	87.2
90.6	96.1	88.5	88.4	91.6
95.3	94.3		88.2	87.0
b	94.2		89.7	
91.9	94.8		88.4	
89.8	*f*		89.9	
90.3	94.5		92.6	
89.2	96.1		89.4	
91.7	93.0		85.8	
88.0	92.4		91.4	
89.1	96.5		90.6	
89.6	94.7			
91.4	94.4		*a*	
93.4	*g*		89.3	
c	88.9		86.9	
89.7	91.9		91.1	
89.6	90.7		87.9	
90.2	90.2		90.9	
88.0	89.9		86.9	
89.5	92.7		91.7	
88.4	92.3		87.2	
89.2	92.3		86.9	
90.3	89.6		89.9	
87.1	91.6		*b*	
91.1	91.9		89.5	
	91.3		88.1	
	89.8		90.2	
	93.6		87.6	
	91.5		89.1	
	91.0		85.1	
	93.0		92.1	
	93.3		86.8	
	90.5		89.8	
	91.6		88.3	
	90.7		*c*	
	93.5		90.6	
	92.4		90.5	
	92.4		89.1	
	89.0		87.9	
	90.5		91.7	
	91.2		87.2	
	91.1		92.3	
	91.2		87.0	
	91.6		90.3	
	91.2		91.3	
	92.6			
	93.4			
	91.7			
	91.5			
	92.5			
	93.4			
	92.9			
	92.1			
	90.9			
	92.3			
	92.7			
	92.4			
	h			
	90.9			
	92.2			
	91.1			
	90.2			
	91.3			
	90.9			
	92.9			
	91.0			
	92.4			
Mean 90.0±0.21	92.3±0.14	89.7±0.36	89.8±0.22	89.7±0.41
Standard deviation 1.7± .15	1.7± .10	1.6± .26	2.3± .15	1.9± .29
Coefficient of variability 1.9± .16	1.8± .10	1.8± .29	2.5± .17	2.1± .32

[a] Number of determinations: Ayrshire, 30; Brown Swiss, 70; Guernsey, 9; Holstein-Friesian, 48; Jersey, 10.

These coefficients for fat are higher, upon the whole, than those reported in the literature. It is likely that this difference may be explained by differences in the methods of experimental procedure employed. In most of the investigations cited, the experimental periods were of one to three days' duration. In many cases the digestive tract may not have been cleared of food residues from diets taken previous to the experimental diet, but instead suitable substances were administered for marking the feces. Probably a more important difference than either of those just mentioned is the fact that in many of the experiments cited, the milk fat was taken as part of a mixed diet. In such cases it is likely that more nonfat substances are extracted from feces by ether than is the case when the diet consists of milk only. In a number of cases this alone might account for the fact that the coefficients for fat recorded in the literature were lower than those reported in this paper.

The apparent digestibility of the total protein was found to be lower than that of the other components studied. (Table 3.) There is some variation in the data, but, on the whole, the results are remarkably uniform. Of the 167 coefficients shown in Table 3, each of which was derived by means of a 10-day digestion trial, only 6 fall below 87 and only 6 exceed 95. The mean of the 167 determinations is 90.9 ± 0.12.

There are small differences in the data obtained for the various breeds. The means of the coefficients of the Ayrshire, Guernsey, Holstein, and Jersey breeds are nearly identical. It would be extremely difficult to obtain results more nearly alike than these with two groups of animals, even if all animals were fed the same kind of milk.

A possible explanation of the fact that the coefficients of apparent digestibility for the protein of Brown Swiss milk are higher than those for the milk of the other breeds is that the values in the first column under Brown Swiss (Table 3) were secured under different conditions from the others. These results were obtained in growth experiments with young rats. The first four coefficients (*d*) were secured with animals weighing 107 to 133 g.[5] During the digestion trial, they consumed 11.8 g protein per 100 g live weight. The next 14 coefficients were obtained with seven animals in two trials. In one trial (*e*, Table 3) their weight ranged from 90 to 119 g (average 101 g) and they consumed 12.8 g protein per 100 g live weight. In the second trial (*f*, Table 3) their weight ranged from 106 to 132 g (average 120 g) and the consumption of protein was 11.8 g per 100 g live weight.

All the other coefficients shown in Table 3 (except the first three for Holstein milk) were obtained with stock animals which weighed, as a rule, from 175 to 300 g. The last nine coefficients under Brown Swiss (*h*) were obtained with stock animals weighing 177 to 321 g (average 235 g.). They consumed 9.6 g protein per 100 g live weight.

In the light of these data, it is reasonable to assume either that the young growing animal, on account of its greater requirement of protein for growth, absorbs the protein of the food more completely than the mature animal, or that, because of its larger food intake in proportion to body weight, it suffers a loss of endogenous nitrogen in the feces which is slightly less in proportion to body weight than is the

[5] g is the abbreviation for gram or grams.

case with the large, mature animal. It is also possible that both factors may be operative, although the first is probably the more important.

Were the 18 coefficients shown under Brown Swiss (Table 3) to be omitted, the mean values for the breeds would agree more closely.

The coefficients shown in Table 3 under (*a*), (*b*), and (*c*) for both the Ayrshire milk and the Holstein milk are those obtained in three successive trials in which the experimental procedure was modified as already explained. In trial (*a*), the feces which showed evidences of urine contamination were washed, but in trials (*b*) and (*c*) no washing was done. Previous to trial (*c*) the animals were clipped. This comparison, although limited in extent, tends to substantiate the soundness of the procedure used in all of the earlier trials.

The apparent digestibility of the total solids of the milk was found to be slightly higher than that of the protein. (Table 4.) As in the case of fat and total protein, the differences between breeds in apparent digestibility of the total solids are so small that it is very probable that no true differences exist. In round numbers, the means of the coefficients for each of three breeds are 92, and for the other two breeds, 93. The other statistical constants also agree closely.

TABLE 4.—*Comparative apparent digestibility of the total solids of the milk of dairy cattle* [a]

	Summary of coefficients of apparent digestibility of total solids				
	Ayrshire	Brown Swiss	Guernsey	Holstein-Friesian	Jersey
	90.6	93.8	90.6	91.7	92.3
	92.0	94.7	92.4	91.0	91.8
	92.5	95.0	92.8	91.2	94.1
	93.7	95.2	92.1	91.8	91.8
	95.0	93.9	94.4	91.8	91.8
	91.9	94.3	91.5	90.9	89.9
	91.5	95.4	90.7	93.1	92.7
	92.6	94.5	92.1	90.9	92.0
	92.0	94.1	92.3	92.0	91.0
	95.8	94.4	90.8	90.6	91.1
		95.2		91.9	
		95.6		93.1	
		93.9		92.5	
		95.4		91.7	
		92.1		93.7	
		92.2		90.2	
		93.7		92.4	
		92.5		90.5	
		92.7		91.2	
		92.7		93.3	
		92.4			
		92.7			
		92.7			
		91.4			
		92.1			
		92.1			
		91.5			
		93.0			
		92.6			
		91.9			
		92.8			
		92.0			
		92.3			
		90.8			
Mean	92.8±0.33	93.3±0.15	92.0±0.23	91.8±0.15	91.9±0.22
Standard deviation	1.5± .23	1.3± .11	1.1± .17	1.0± .10	1.1± .16
Coefficient of variability	1.6± .25	1.4± .12	1.2± .18	1.1± .11	1.1± .17

[a] Number of determinations: Ayrshire, 10; Brown Swiss, 34; Guernsey, 10; Holstein-Friesian, 20; Jersey, 10.

The feces of 14 rats fed Brown Swiss milk and of 10 rats fed Holstein milk were tested carefully for sugar, both Fehling's and Benedict's methods being used. All these tests were negative. Similar results had been secured in previous trials with two brands of evaporated milk and two brands of dried milk (*19*, *20*). It is believed, therefore, that the sugar of fresh whole milk is completely digestible.

The fat, protein, and total solids of the milk fed during the digestion trials are shown in Table 5. Each value given represents the average of two or more determinations made upon a composite sample.

TABLE 5.—*Composition of milk fed during digestion trials*

Breed	Fat	Protein	Total solids	Breed	Fat	Protein	Total solids
	Per cent	*Per cent*	*Per cent*		*Per cent*	*Per cent*	*Per cent*
Ayrshire	3.04	----------	12.15	Guernsey	----------	3.70	13.79
	----------	3.35	12.15		4.35	----------	14.02
	----------	3.53	12.21		----------	2.95	10.60
	----------	3.26	12.64		----------	2.97	10.34
	3.90	----------	12.17		----------	3.26	11.75
	2.99	----------	12.47	Holstein	2.93	----------	11.65
	2.35	3.65	11.80		2.77	----------	11.28
	----------	3.86	13.62		----------	2.99	11.15
	----------	3.79	13.72		----------	2.93	11.04
	----------	3.89	13.84		----------	2.76	11.02
Brown Swiss	4.02	3.91	13.89	Jersey	----------	3.64	13.74
	4.33	3.88	14.12		5.08	----------	14.96
	2.83	3.44	12.34				
	2.69	3.50	11.66				
	----------	4.14	13.60				
	----------	4.15	13.05				
	3.29	----------	13.22				
	3.08	----------	11.74				

SUMMARY

The apparent digestibility of the fresh whole milk of five breeds of dairy cattle was studied by means of 10-day digestion trials with albino rats. Milk only was fed.

The fat of fresh whole milk was found to be very highly digestible, the coefficients obtained indicating an apparent digestibility of about 99 per cent. There were no significant differences in digestibility of the milk fat of the breeds studied.

The protein of fresh whole milk was found to be about 91 per cent digestible. The data show slight differences between the milks of the breeds, but on account of some variations in the methods employed at different times in the course of the investigation, these differences between breeds are believed to have no significance.

The total solids of fresh whole milk were slightly higher in digestibility than the protein, as would be expected, but no true differences between breeds were found.

The sugar of fresh whole milk was found to be completely digestible.

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THE CARBON METABOLISM OF FUSARIUM OXYSPORUM ON GLUCOSE [1]

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INTRODUCTION

A very important phase of research in plant pathology is that which deals with the physiological relationships existing between the causative organism and the host. Any information on the biochemistry of the causative organism should prove of value in studying the physiology of a plant disease. It is very probable that some metabolic product of the organism is the important factor producing the symptoms of the disease in the plant.

REVIEW OF LITERATURE

In the past considerable work has been done on the biochemistry of *Fusarium lini* Bolley, the organism causing flax wilt. In 1920 Tochinai (*9, 10, 11*)[2] reported a rather complete study of the food requirements of this organism. In 1924 Anderson (*1*) repeated much of the work of Tochinai and also studied the products of metabolism of this organism on glucose and xylose media. He found that the organism produced a rather typical alcoholic fermentation on glucose. About 90 per cent of the orginal carbon could be accounted for in the products of metabolism studied. In the case of xylose about 80 per cent of the original carbon could be accounted for in the ethyl alcohol, carbon dioxide, and mycelium produced. He suggested the possibility that the alcohol caused the wilt.

In 1926 Letcher and Willaman (*6*) attempted to correlate the pathogenicity of various strains of *Fusarium lini* with their ability to produce ethyl alcohol. They examined nine strains of the fungus and found that the two which were the least virulent on flax produced the least alcohol. It should be noted, however, that the strain which produced the most alcohol was not the most virulent.

In 1928 White and Willaman (*14, 15*) studied the fermentation of pentoses by *Fusarium lini* and accounted for nearly 100 per cent of the original carbon in the form of carbon dioxide, ethyl alcohol, and mycelium. They also reported the growth of the organism on the postulated intermediate products formed by yeast in the pyruvic acid theory of fermentation. They believed that *F. lini* ferments glucose by the same mechanism as that of yeast.

Reynolds (*8*) in 1926 reported on the utilization of various carbohydrates and nitrogen compounds by *Fusarium lini*.

In 1931 Birkinshaw, Charles, Raistrick, and Stoyle (*2*) reported on the carbon metabolism of various species of Fusarium, including *F. oxysporum*, grown on a glucose medium. They found some variation among the various species but all of them produce ethyl alcohol.

[1] Received for publication July 22, 1932; issued April, 1933.
[2] Reference is made by number (italic) to Literature Cited, p. 481.

PURPOSE OF THE INVESTIGATION

The purpose of the present work has been to open the way for an investigation of another wilt-producing fungus, *Fusarium oxysporum* Schlecht., using the same type of medium and employing essentially the same methods of determining the products of metabolism as were employed by Anderson (*1*) on *F. lini*. This has made it possible to compare the metabolism of these two organisms under practically the same conditions. The principal part of the study has been to determine the quantitative distribution of the chief products of metabolism (ethyl alcohol, carbon dixoide, and mycelium) of *F. oxysporum* on glucose. A further study has been made to determine whether or not ethyl alcohol, in concentrations equal to that produced by *F. oxysporum* in artificial cultures, is toxic to potato cuttings.

METHODS

SOURCE OF FUSARIUM OXYSPORUM CULTURE

The culture of the organism used in this work was obtained from the mycological herbarium of the University of Minnesota through the courtesy of Dr. Louise Dosdall. This was isolated in 1919 by Dr. G. R. Bisby (*3*) in connection with his studies on the Fusarium diseases of potatoes and truck crops.

CULTURE MEDIA

The mineral medium used in this work was the same as that used by Tochinai (*9*) and Anderson (*1*) in their work on *Fusarium lini*. It has the following composition:

Ammonium nitrate	1.00 g[2]
Magnesium sulphate	.25 g
Monopotassium phosphate	.50 g
Water to make	1,000 c c

To this stock solution sufficient glucose was added to make an approximately 2 per cent solution of glucose. The exact quantity of glucose was determined after sterilization by the Folin-Wu (*5*) method.

HYDROGEN-ION CONCENTRATION

Hydrogen-ion concentration determinations were made by the electrometric method using the quinhydrone electrode.

CULTURE FLASKS AND TEMPERATURE OF INCUBATION

The culture flasks used were 500 c c Erlenmeyer flasks fitted with 2-hole rubber stoppers. Glass tubes were placed through the holes of the stoppers and bent at right angles just above them. One of the tubes extended just below the stopper and the other reached to the bottom of the flask. The outer end of each tube was plugged with cotton and fitted with a rubber tube that could be closed by a pinchcock. Into each flask 300 c c of the medium were placed and the long tube was drawn up so that the lower end was about an inch above the surface of the liquid. The flasks were then sterilized at 15 pounds pressure for 20 minutes. When cooled each flask was inoculated by the addition of 5 c c of a spore and mycelium suspension of the

[2] g is the abbreviation for gram or grams recently adopted by the Style Manual for U. S. Government printing.

organism in water. The stoppers were then adjusted and sealed with paraffin and the rubber connections closed with pinchcocks.

The flasks were kept at room temperature in a laboratory where the temperature varied only slightly from 25° C.

DETERMINATION OF CARBON DIOXIDE

Carbon dioxide was determined at frequent intervals throughout the experiment in order to prevent loss of this product of metabolism. The method used was to aerate each culture flask into barium hydroxide solution contained in a Truog (*12*) tower fitted into a 500 c c suction flask through a rubber stopper. The air used for aeration was freed from carbon dioxide by passing it through soda lime. In order to prevent loss of alcohol, a Truog tower containing concentrated sulphuric acid was placed ahead of the barium hydroxide tower. A separate sulphuric acid tower was prepared for each culture flask. These were used every time a culture flask was aerated for carbon dioxide and finally the alcohol was determined, as will be described later. After aeration the excess of barium hydroxide was titrated with standard hydrochloric acid solution using phenolphthalein as an indicator. Before flasks were removed for final analysis the long tube was pushed below the surface of the liquid and aerated for 30 minutes in order to remove the last traces of carbon dioxide. Each time the flasks were aerated for carbon dioxide a blank determination was made. The titer of the carbon dioxide determinations was subtracted from this blank and the difference used in calculating the carbon dioxide in the sample.

DETERMINATION OF DRY MATTER AND CARBON IN THE MYCELIUM

When the experiment was started a sufficient number of culture flasks were prepared so that duplicate samples could be analyzed at intervals of a week or 10 days over a period of about two months. The weight of mycelium was determined by filtering through a weighed Gooch crucible and washing with water. The pad of mycelium was then dried to constant weight at 100° C. The filtrate was made up to a volume of 500 c c and aliquot portions were used for subsequent determinations.

The carbon in the mycelium was determined by the wet combustion method using the Knorr apparatus and the details of procedure as outlined by White and Holben (*13*). The entire mycelium mat, together with the asbestos, was introduced into the digestion flask.

DETERMINATION OF ALCOHOL

For the determination of ethyl alcohol the method of Dox and Lamb (*4*) was used with modifications. In this method the alcohol is oxidized to acetic acid which is distilled and titrated with standard alkali. After filtering and washing the mycelium and making up the filtrate to a volume of 500 c c, a 300 c c aliquot of the filtrate was saturated with solid ammonium sulphate and the mixture aerated for 48 hours into a Truog tower containing concentrated sulphuric acid. The acid mixture, together with the glass beads, was then transferred to a 2-liter Claison flask containing 22 g of potassium dichromate. After standing for 20 minutes the resulting acetic acid was distilled and titrated with standard alkali. In this distillation the flask was

heated until foaming occurred. Next 100 c c of carbon-dioxide-free water were added to the distilling flask, the distillation was repeated, and the distillate titrated. This last procedure was repeated until a constant titer was obtained. This represents the amount of sulphuric acid unavoidably distilled at each distillation, and the constant titer times the number of distillations represents a blank which must be subtracted from the sum of all the titrations.

Since in the determination of carbon dioxide a sulphuric acid tower was always placed ahead of the barium hydroxide tower to collect any alcohol given off, another alcohol determination was made on the contents of this tower. The quantity of alcohol found was added to that found in the filtrate to give the total alcohol produced in a given flask.

DETERMINATION OF GLUCOSE

Glucose was determined by the method of Folin and Wu (*5*). An aliquot of the filtrate from the mycelium determination was diluted so that 1 c c contained about 0.1 mg of glucose. Under these conditions the color of the unknown matches closely that of the Folin and Wu dilute glucose standard.

PRESENTATION OF DATA

Sixteen flasks with 300 c c of medium containing a known quantity of glucose as the only source of carbon were inoculated with a spore and mycelium suspension of *Fusarium oxysporum*. These were stoppered and the aeration tubes were closed by means of rubber tubes and pinchcocks in order to prevent loss of carbon dioxide. Carbon dioxide was determined at frequent intervals. During the active growth of the organism it was determined daily. At intervals of a week or 10 days two flasks were removed and analyzed for residual carbon dioxide, carbon in the mycelium, glucose, and ethyl alcohol. The original culture medium was analyzed for glucose and the pH determined. The last two flasks were used for pH determinations.

TABLE 1.—*Distribution of metabolic products of Fusarium oxysporum on a glucose medium at various stages of growth*

Age of culture (days)	Mycelium				Carbon dioxide		Ethyl alcohol		Glucose		Total recovery of carbon as related to initial carbon [a]	pH
	Weight	Weight of carbon	Carbon	Carbon as related to initial carbon [a]	Weight of carbon	Carbon as related to initial carbon [a]	Carbon in 300 c c	Carbon as related to initial carbon [a]	Carbon in 300 c c	Carbon as related to initial carbon [a]		
	Gram	*Gram*	*Per cent*	*Per cent*	*Gram*	*Per cent*	*Gram*	*Per cent*	*Gram*	*Per cent*	*Per cent*	
0									2.0633	100	100	4.15
12	0.1399	0.0651	46.53	3.15	0.1998	9.68	0.3501	16.97	1.6170	78.37	108.17	.....
20	.2466	.1129	45.78	5.47	.5150	24.96	1.0153	49.21	.0141	.68	80.33	.....
27	.2432	.1095	45.02	5.31	.5389	26.12	1.0172	49.30	.....	.....	80.72	.....
36	.2484	.1140	45.89	5.52	.5457	26.45	[b]1.0379	50.30	.....	.....	82.23	.....
46	.2440	.1118	45.82	5.42	.5779	28.01	[b]1.0707	51.89	.....	.....	85.32	.....
59	.2399	.1204	50.18	5.83	.6376	30.90	1.0030	48.61	.....	.....	85.34	.....
68	.1929	.1027	53.24	4.98	.7051	34.17	.9878	47.88	.....	.....	87.03	6.55

[a] Expressed as percentage of the carbon in the glucose originally present.
[b] 1 determination.

In all, four experiments were run. In Table 1 and Figure 1 the data are given for one representative experiment. It should be noted that results are expressed in terms of carbon and also in terms of percentage of the carbon of the glucose originally present in each flask.

Referring to Table 1 and Figure 1, the following facts are brought out concerning the progressive changes in the products of metabolism.

THE MYCELIUM AND ITS CARBON CONTENT

The weight of mycelium increases rapidly, reaches a maximum at 36 days, and finally decreases. The maximum is reached at the time that all the glucose has been consumed. Apparently stored material

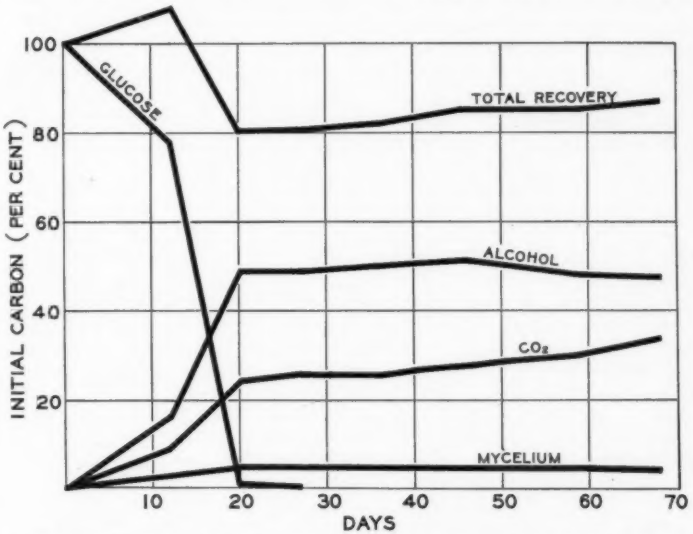

FIGURE 1.—Distribution of metabolic products of *Fusarium oxysporum* when grown on glucose

in the mycelium is consumed after other sources of food have been utilized.

The weight of dry mycelium produced approximates that found by Anderson (*1*) for *Fusarium lini*, although he used only one-third as much medium. He found as high as 16.75 per cent of the original carbon in the mycelium, while with *F. oxysporum* 5.83 per cent is the maximum. *F. lini* shows a very definite utilization of ethyl alcohol for mycelium production. *F. oxysporum* is apparently unable to utilize ethyl alcohol for mycelium building purposes.

The percentage of carbon in the mycelium remains very constant at about 46 per cent for 46 days when it increases to a final value of over 53 per cent. This increase in carbon content of mycelium with age was also noted by Anderson (*1*) in his work on *Fusarium lini*. Since carbohydrates contain about 40 per cent of carbon, proteins from 51 to 55 per cent, and a typical fat such as tristearin 76.87 per cent, Anderson believes that the increase of carbon in the mycelium indicates

a change of the carbohydrate in young mycelium into fat as the mycelium ages. Since the percentage of nitrogen in the mycelium decreased with age, he felt there was not a change of carbohydrate to protein. Although nitrogen was not determined on the mycelium in this work on *F. oxysporum*, it is a reasonable assumption that the increase in carbon content is due to a conversion of carbohydrate into fat.

The percentage of original carbon found in the mycelium is well over 5 during active growth. In the last analysis this drops to 4.98 per cent, which indicates a utilization of carbon in the mycelium for food purposes as other sources of food are consumed.

CARBON DIOXIDE

Carbon dixoide is produced throughout the experiment. During the early part of the experiment carbon dioxide was produced very rapidly, necessitating its determination daily. When the glucose is all consumed, the rate of carbon dioxide production decreases so that it is necessary to determine it only at infrequent intervals. The percentage of original carbon in the form of carbon dioxide increases throughout the experiment, reaching a maximum of 34.17 per cent in the final analysis.

ETHYL ALCOHOL

Ethyl alcohol is produced rapidly by *Fusarium oxysporum*. In 20 days the percentage of original carbon in the form of ethyl alcohol is 49.21. This increases gradually to 51.89 per cent at the end of 46 days. From then on the quantity of alcohol decreases, falling to 47.88 per cent of the original carbon at the end of the experiment. Anderson (*1*) in his work on *F. lini* found that this organism converted 52.75 per cent of the original carbon into alcohol when the amount of alcohol was at a maximum. At the end of the experiment the original carbon in the form of ethyl alcohol was only 29.51 per cent. *F. lini* very definitely utilizes ethyl alcohol as a food material. There is only slight utilization of ethyl alcohol by *F. oxysporum*.

GLUCOSE

The glucose of the medium is consumed rapidly. In 20 days there is only a trace left. Since the total recovery of carbon at the end of the 12-day period is 108.17 per cent, it is felt that the error is in the determination of glucose. It is very possible that in the early stages of growth the organism converts glucose into intermediate products which have the power of reducing copper solutions. If this is true, the value for glucose at the 12-day period is high.

TOTAL RECOVERY OF CARBON

In the column headed "total recovery" in Table 1 are recorded the sum of the values obtained for the various products of metabolism. It will be noted that at the end of the 12-day period the recovery is 108.17 per cent. As pointed out above this is likely due to the production of intermediate products which reduce copper solutions, rendering the glucose determination high. At the end of the 20-day period the total recovery has fallen to 80.33 per cent. From then on the total recovery gradually increases until at the end of the experiment it is 87.03 per cent. The fact that all of the carbon is not

accounted for indicates that there are other products of metabolism than those studied, although some of the loss may be due to incomplete recovery of some of the products studied.

HYDROGEN-ION CONCENTRATION

Determinations of pH were made only at the beginning and at the end of the experiment. The initial pH was 4.15 and the final pH was 6.55. Anderson (*1*) in his work on *Fusarium lini* found that this organism changed the pH of the medium toward the optimum for growth. It appears that the same occurs with *F. oxysporum*.

RATIO OF PRODUCTS OF METABOLISM TO ONE ANOTHER AND TO GLUCOSE CONSUMED

The relationships existing between the products of metabolism and the compounds consumed by a fungus have been expressed by means of various ratios and percentages. Such terms as respiration coefficient, economic coefficient, respiration equivalent, plastic equivalent, etc., have been introduced into the literature. There has been such a lack of uniformity with regard to the usage of these terms that in Table 2 a formula is placed at the head of each column to make perfectly clear the sense in which these terms are used in this discussion. They are used in the same sense as used by Peterson, Fred, and Schmidt (*7*).

The respiration coefficient represents the grams of carbon dioxide produced per gram of dry mycelium. It will be noted from Table 2 that this coefficient gradually increases with the age of the culture. Finally there is a rapid increase due to the consumption of the mycelium itself as a food material. The values are very much higher than those found by Anderson (*1*) for *Fusarium lini*.

TABLE 2.—*Quantitative relationships existing between the various metabolic products of Fusarium oxysporum when grown on a glucose medium*

Age of culture (days)	Respiration coefficient, $\frac{\text{weight of } CO_2}{\text{weight of mycelium}}$	Economic coefficient, $\frac{\text{weight of sugar consumed}}{\text{weight of mycelium}}$	Respiration equivalent, $\frac{\text{carbon of } CO_2 \times 100}{\text{carbon consumed}}$	Plastic equivalent, $\frac{\text{carbon of mycelium} \times 100}{\text{carbon of glucose consumed}}$	Alcohol equivalent, $\frac{\text{carbon of alcohol} \times 100}{\text{carbon of glucose consumed}}$	Carbon of alcohol / carbon of CO_2
12	5.24	7.98	44.76	14.60	78.45	1.75
20	7.66	20.78	25.03	5.51	52.09	1.97
27	8.13	23.32	26.12	5.31	49.30	1.88
36	8.06	24.79	26.45	5.52	50.30	1.90
46	8.68	23.24	28.01	5.41	51.90	1.85
59	9.75	25.66	30.90	5.83	48.61	1.57
68	13.40	31.92	34.17	4.98	47.90	1.40

The economic coefficient represents the grams of glucose necessary to produce 1 g of dry mycelium. This coefficient increases with the age of the culture, reaching a maximum of 31.92 in 68 days. Anderson (*1*) found a maximum value of 13.1 with *Fusarium lini*.

The respiration equivalent is the percentage of carbon consumed that is transformed into carbon dioxide. With the exception of the 12-day period, where the value is high due to a false sugar value, this

equivalent increases with age. Anderson (*1*) found slightly higher values for *Fusarium lini*.

The plastic equivalent represents the percentage of carbon source used in mycelium synthesis. Here again is found a high value at the 12-day period due to a false sugar value. The other values are approximately constant but lower than those found by Anderson (*1*) for *Fusarium lini*.

The alcohol equivalent is a new term that the authors have introduced which expresses the percentage of carbon of the glucose consumed that is converted into alcohol. At the end of the 12-day period the value is 78.45 which is undoubtedly too high due to a false glucose value as mentioned above. For the remaining periods the value drops to around 50. In the last two periods the value drops due to consumption of the alcohol by the fungus.

In the last column the ratio of carbon in alcohol to carbon in carbon dioxide is given. In a typical alcoholic fermentation glucose is decomposed according to the following equation:

$$C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2.$$

In this equation the ratio of carbon in alcohol to carbon in CO_2 is 2:1. It will be seen from Table 2 that at the end of the 20-day period the value is 1.97:1, which is very close to the theoretical for a typical alcoholic fermentation. From this point on the ratio decreases until at the end of the experiment it is 1.40:1. Here again utilization of the alcohol by the fungus is indicated.

In the early part of this study an error was made in making up the nutrient mineral solution. In place of 1 g of NH_4NO_3 per liter, 100 g per liter were used. It is felt worthy of mention that *Fusarium oxysporum* grew on this medium and produced a typical alcoholic fermentation. When the distribution of the products of metabolism were charted as in Figure 1, in general, the curves were the same. The main difference between the two figures was that the rate of production of the various products of metabolism on the strong mineral solution was about one-third as fast as on the weak mineral solution.

THE EFFECT OF ALCOHOL ON POTATO CUTTINGS

Anderson (*1*) has suggested that the toxicity of *Fusarium lini* to flax may be due to the production of ethyl alcohol by the organism in the plant tissue. When it was ascertained that *F. oxysporum* produces ethyl alcohol on glucose it became of interest to study the effect of solutions of ethyl alcohol at various concentrations on potato cuttings.

Solutions of alcohol were made, varying in concentration from 0.5 to 5 per cent in increments of 0.5 per cent. Potato cuttings were placed in these solutions in beakers. Several cuttings were placed in tap water as a control. In no case was there any appreciable change within 24 hours, other than a slight curling of the leaves. After 48 hours a mottled yellow began to appear on the leaves of the cuttings in alcohol. This did not occur in the controls. The stems remained firm. When the cuttings in alcohol were shown to a pathologist familiar with potato diseases, he believed they were affected with an extreme case of leaf mosaic. There was no wilting of the stems even

at the end of five days. A very striking odor resembling that of ripe cantaloupes was given off by the cuttings in alcohol.

Since in all of the above work the stems remained firm, it was decided to determine the concentration of alcohol necessary to produce definite and rapid wilting. Cuttings were placed in solutions containing from 10 to 55 per cent of alcohol in increments of 5 per cent. Within two hours there was a general wilting in all cases, the wilting increasing in severity with the higher concentrations. There was no appreciable change in color of the leaves as was noted with the more dilute solutions of alcohol. The cantaloupe odor previously noted was very strong in all cases. Judging from the esterlike odor, one is led to the hypothesis that the potato plant is able partly to protect itself against ethyl alcohol by converting it to an ester.

The findings in solutions of alcohol up to 5 per cent are in agreement with the work of White (*16*) who observed no ill effects on tomato cuttings in solutions of ethyl alcohol up to 4 per cent.

SUMMARY AND CONCLUSIONS

It is evident from the data presented that the main products of metabolism of *Fusarium oxysporum* on glucose are carbon dioxide and ethyl alcohol. The proportion of these two compounds formed indicates that the organism causes a rather typical alcoholic fermentation. In this respect *F. oxysporum* is similar to *F. lini*.

Fusarium lini very definitely uses ethyl alcohol as a source of food supply while *F. oxysporum* makes only slight use of this product.

Work with potato cuttings in ethyl alcohol solutions would indicate that this product is not responsible for the wilting of potato plants. It is likely that small quantities of alcohol are rendered nontoxic by conversion into an ester by the potato plant. Potato cuttings in a 10 per cent alcohol solution showed very definite symptoms of wilting. It is quite possible that in this case the concentration of alcohol in the tissue was much less than 10 per cent. Although there is no experimental evidence to support the theory that alcohol is responsible for the wilting, it is reasonable to suppose that alcohol may at least be a factor in the production of the symptoms of wilt in potato plants infected with *Fusarium oxysporum*.

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